A STUDY OF THE RELATIONSHIPS BETWEEN ANAEMIA AND CERTAIN GASTRO-INTESTINAL DISEASES

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SUMMARY

Gastritis and abnormalities of gastric secretion are common in patients with iron deficiency anaemia, but the mechanism of these gastric changes is unknown. The present studies on the rat and the human explore more fully the effects of iron deficiency on the gastrointestinal tract. In addition, the interrelationships between pernicious anaemia, iron deficiency, gastric carcinoma and oesophageal carcinoma have been investigated. All these studies have involved secretory, histological and haematological investigations on groups of patients together with tests for serum antibodies to parietal cells and intrinsic factor.

A method of inducing severe iron deficiency anaemia in the rat was developed. Pregnant female rats were placed on an iron deficient diet and after gestation, the infant rats were maintained on the same iron deficient diet and killed 28 and 42 days after birth. Retarded growth and severe iron deficiency anaemia with haemoglobin levels ranging between 1.0 and 2.1 g% were observed. In these rats, histological and histochemical studies of the liver revealed severe central zone damage with a decrease in succinic dehydrogenase and an increase in fat in

these areas. The activity of other enzymes was not depressed. There were only minimal or inconsistent histological and histochemical abnormalities in the stomach, small intestine and colon. Gastric acid studies were carried out on pylorus ligated rats with iron deficiency anaemia prepared by the above method. Impairment of gastric acid secretion was not demonstrated in these rats.

Gastric function was studied by means of the augmented histamine secretion test and histamine infusion test in 17 patients presenting with moderate to severe iron deficiency anaemia. In those patients with idiopathic iron deficiency anaemia, there was a high incidence of achlorhydria and gastritis with circulating parietal cell antibodies in the peripheral blood. None of these patients had antibodies to intrinsic factor. In those patients in whom anaemia was due to chronic blood loss, the gastric secretion of hydrochloric acid was reduced but was not usually absent. Gastric parietal cell antibodies could not be detected. The acid secretion in some patients had increased when they were retested several months after the correction of anaemia. This improvement in secretion was seen also after treatment in patients with anaemia due to folic acid deficiency. The stimulation of

patients prior to correction of anaemia with two doses of histamine, 0.04 mg/kg and 0.1 mg/kg showed a greater acid response to the latter dose. It is suggested that patients with iron deficiency anaemia and gastritis, may be divided into two groups depending on whether the gastric mucosal lesion precedes or follows the iron deficiency anaemia.

Seventy patients with gastric carcinoma were investigated. In 54, pentagastrin or augmented histamine stimulation tests of gastric secretion were performed. Intrinsic factor production was studied in 40 cases. No statistically significant differences were found between patients with body or antral growths with regard to acid or intrinsic factor. However, some patients with antral tumours had normal or increased acid production. Some patients who secreted acid had a low intrinsic factor output but there was no evidence of inhibitors in the gastric juice of these patients.

The incidence of parietal cell and intrinsic factor antibodies was studied in the 70 patients. In addition to 4 known cases of pernicious anaemia, a further 4 overt or latent cases were found increasing the incidence of pernicious anaemia in the series from 5.6% to 10.4%. In some other patients the serum vitamin B_{12} level was low for no obvious reason and in

these cases serum inhibitors to the growth of Euglena gracilis were not demonstrated.

Ninety-two consecutive cases of oesophageal squamous carcinoma were studied. Eight of these patients had undergone a previous gastric operation, either a partial gastrectomy or gastroenterostomy. On average the gastric operation had been performed 19 years previously. Six of the 8 cases of tumour occurred in the lower oesophagus. In contrast, only one case of achalasia was found, and one case of Paterson-Kelly syndrome. Five of the 92 patients had thyroid disease. The interval between the development of the thyroid disorder and the tumour varied from 6 to 37 years with a mean of 15 years. The mean age of this group of patients was the same as that of the whole group of 92 patients. Sixteen of the 92 patients had parietal cell antibodies and the incidence was 17% for upper middle and lower oesophageal tumours. The 27% incidence in female patients was no greater than in a control group.

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SECTION I - IRON DEFICIENCY, GASTRITIS AND GASTRIC SECRETION IN THE HUMAN AND IN THE RAT

INTRODUCTION

Iron Deficiency, Gastritis and Gastric Secretion in the Human

The inter-relationship between pernicious anaemia and idiopathic hypochromic anaemia was first commented upon in 1924 by Faber and Gram and in 1932 by Heath who reviewed the situation at that time and described a family in which both conditions occurred singly or in combination. Heath concluded that pernicious anaemia was secondary to the absence of a 'specific factor' in the stomach. He believed that idiopathic hypochromic anaemia was primarily the result of a deficiency conditioned by a disorder of the gastrointestinal tract leading to an inability to absorb or utilise haemoglobin building material from the food. It is only now that we can explain these findings on a more scientific basis.

There is evidence that in any group of iron deficient patients there is a greater incidence of gastritis and gastric atrophy than in a comparable control group. For example, 74% in iron deficient patients as compared to 29% in controls (Davidson and Markson 1955). Similarly Badenoch, Evans and Richards (1957) found abnormalities in 86% and Coghill and Williams (1958) in 79% of iron deficient patients. The significance of the gastritis or atrophy is uncertain and although all degrees of gastric mucosal change from superficial gastritis to complete gastric

atrophy have been noted in association with the chronic iron deficiency anaemia there is little histological evidence of any reversal of these changes following iron therapy (Davidson and Markson 1955 ; Lees and Rosenthal 1958 ; Baldini and Cheli 1957), except for the one case described by Badenoch, Evans and Richards (1957).

Most people now agree that the secretion of hydrochloric acid is depressed in the presence of chronic iron deficiency anaemia and that there is an increased incidence of complete achlorhydria (Faber 1909 ; Witts 1930 ; Davidson and Markson 1955 ; Badenoch, Evans and Richards 1957 ; Bock, Richards and Witts 1963 ; Dagg, Goldberg, Anderson, Beck and Gray 1964). Many of the earlier workers, for example Leonard (1954), assessed achlorhydria by using a smaller than "maximal" dose of histamine. He found that 43% of patients with iron deficiency were achlorhydric. In the same way, Badenoch, Evans and Richards (1957) found 54% and Beveridge, Bannermann, Evanson and Witts (1965) found 40%. Using the 0.04 mg./kg. body weight dose of histamine, the percentage of patients with achlorhydria is less, for example 30% (Jacobs, Lawrie, Entwistle and Campbell 1966) and 16% (Callender, Retief and Witts 1960).

There is a much greater difference of opinion as to the reversibility of this depressed gastric acid secretion. Badenoch, Evans and Richards (1957) stated that the secretion of acid often improved with the correction of the iron deficiency and one of their patients with achlorhydria was said to have a return of acid. Davidson and Markson (1955) claimed that there was a return of acid in two patients with achlorhydria associated with superficial gastritis but not in two others in whom the achlorhydria was associated with gastric atrophy. Leonard (1954) claimed to have

demonstrated the reversibility of achlorhydria in several cases but Lawrie, Smith and Forrest (1964) using the histamine infusion test, found no significant improvement of acid secretion after therapy, and Vaish, Sampathkumar, Jacob and Baker (1965) had similar findings in patients with tropical sprue who had depressed acid secretion. There is, however, some evidence that patients can in fact improve their acid secretion in gastritic states, for example, Bock and Witts (1963) showed a considerable increase in acid secretion following the treatment of thyrotoxic patients and Vaish, Sampathkumar, Jacob and Baker (1965) showed that one patient with tropical sprue who had not become achlorhydric increased tha amount of acid that he secreted. Spontaneous recovery of gastric secretion has been demonstrated also (Weir 1957).

Antibodies to gastric parietal cells have been detected with greater frequency in iron deficient patients than in control populations. For example, Markson and Moore (1962) found complement fixing antibodies against gastric mucosa in 6 out of 34 iron deficient patients, whereas no antibodies were found in matched controls. Dagg, Jackson, Curry and Goldberg (1966) found a 23% incidence in 114 iron deficient patients ; and Jacobs, Lawrie, Entwistle and Campbell (1966) found a 38% incidence in 44 patients. In addition, Dagg, Goldberg, Anderson, Beck and Gray (1964) noted that these iron deficient patients with antibodies were likely to be achlorhydric.

The Effects of Iron Deficiency on the Gastrointestinal Tract of the Rat

While it is generally accepted that in the human there is an association between chronic iron deficiency and atrophic gastritis, the induction of iron deficiency in animals has failed to produce significant gastric lesions (Valberg, Taylor, Witts and Richardson (1961) ; Binder, Fischer, Thayer, Spencer and Spiro 1966). This may have been due to failure to induce severe iron deficiency and/or the short duration of the experiments. For example, in the study by Binder, Fischer, Thayer, Spencer and Spiro (1966), the iron deficient diet was commenced after birth and the animals were killed at 70 or 130 days. The lowest haematocrit obtained was 33%, there was no impairment of growth and no histochemical change could be demonstrated in the gastric mucosa. If iron deficiency does cause changes in the gastric mucosa, a possible mechanism would be a depletion or alteration of the iron containing enzymes. It is generally accepted that in the iron deficient state, the synthesis of haem enzymes is impaired but there is no consistent pattern of change for all the tissues of the body or for all the enzymes involved. For example, in the rat, liver and kidney cytochrome C become depleted early in the induction of iron deficiency (Beutler 1957). Impaired succinic dehydrogenase activity is found in the myocardium but not in other organs. The turnover of haem proteins synthesised intracellularly is slow and cells which are depleted in these enzymes may require to be replaced by new cells before the enzyme content of the organ or mucosa is increased. For example, the depletion or repletion of cytochromes in the intestinal

mucosa of iron deficient rats (Dallman and Schwartz 1965) and children (Dallman, Sunshine and Leonard 1967) depends on cell renewal. In the human, a depletion of cytochrome oxidase in epithelial cells in iron deficiency has been widely observed but there is no correlation between this reduction and the presence of atrophic gastritis (Jacobs 1961) and the same enzyme reduction can be found in sideropenic patients without anaemia (Dagg, Jackson, Curry and Goldberg 1966). Iron deficiency may have metabolic effects in sites other than the gastrointestinal mucosa. For example the increased incidence of megaloblastic anaemia in iron deficient pregnant women suggests a defect in folate metabolism (Chanarin, Rothman and Berry 1965). In addition, iron deficient patients may have an increased urinary excretion of formamino-glutamic acid after a histidine load (Chanarin, Bennett and Berry 1962).

In the rat, iron deficiency can lead to a decrease in liver formamino - transferase activity (Vitale, Restrepo, Valez, Riker and Hellerstein 1966). Other enzyme changes have been reported in rat liver in iron deficiency, for example reductions in xanthine oxidase, glucose 6-phosphate dehydrogenase, glutamic - oxaloacetic transaminase and phosphoglucomutase.

In the present studies, a method for inducing severe iron deficiency in the rat was developed and histological and histochemical studies were performed on the gastrointestinal tract of such animals. In the human, the interrelationships between iron deficiency anaemia and pernicious anaemia and the function and histology of the stomach

in iron deficiency anaemia have been studied. Assessments are made of the recovery in function after the correction of the deficiency or anaemia.

MATERIAL AND METHODS

Animal Studies

Female rats (CD strain, Charles River Laboratories, U.S.A.) derived originally from Sprague Dawley stock were used. In some cases, the animals obtained were 7 days pregnant. All animals were maintained in plastic cages and received demineralised water. Age and weight controls were used.

Diet

A commercial diet supplied by the Nutritional Biochemical Company was employed. The iron content in this diet was less than one part per million. When such a diet was supplemented by 50 mg. of iron per Kg., it was associated with normal growth in rats as well as a reversal of anaemia in rats already rendered iron deficient.

Haematological Studies

At the time of killing, blood was taken from the heart and citrated for haemoglobin estimation. In some animals which had received the iron deficient diet, blood was also taken for estimates of serum vitamin B_{12} and folate.

Acid Studies

Some groups of iron deficient and control rats were subjected to measurements of gastric acid secretion. Before the test, the animals were fasted for 48 hours, being allowed normal saline only. They were then weighed and anaesthetised with ether and a standard Shay, Sun,

and Gruenstein (1954) pyloric ligation performed. Three or four hours later they were killed by means of a blow on the head. The animal was reopened, the lower oesophagus clamped and the whole stomach removed. Measurements were made on the total volume of gastric juice, its pH and the total mEq of acid. Recording was made of the presence of blood or food in the gastric juice.

Histology and Histochemistry

Specimens of glandular stomach, proximal and distal small intestine, colon and liver were obtained from animals killed by a blow on the head. Each specimen was divided into two, one part was fixed in 10% neutral buffered formaldehyde solution and the other part was frozen immediately in liquid nitrogen and stored at -70°C in plastic bags. The specimen fixed in formaldehyde solution was embedded in paraffin, sections were cut and stained with haematoxylin and eosin. Sections of liver were also stained with periodic acid fuchsin, Prussian blue, flamingo red and Sudan black stains.

Sections for histochemistry were cut at approximately 4 μ in a cryostat at -20°C, delivered on to a cover slip and according to the enzyme method to be used, either allowed to thaw and dry briefly at room temperature, or fixed in cold neutral phosphate buffered 10% formalin or cold acetone.

Microsomal enzymes

Non-specific esterase was demonstrated by the indoxyl method of Holt (1958) using sections fixed in formalin with incubation at 37°C

for 45 minutes.

Cytochrome oxidase was measured by the method of Burstone (1961) using p-aminodiphenylamine and variamine blue B base with incubation for 60 minutes at 37°C.

Membrane associated enzyme

Leucine aminopeptidase. This was measured by the method of Nachlas, Crawford and Seligman (1957) using unfixed sections and 1-leucyl-B-naphthylamide as the substrate with incubation at 37°C for 15 minutes.

Alkaline phosphatase was measured by the method of Burstone (1958) using naphthol AS-TR phosphate as the substrate and fast red TR as the diazonium salt with sections fixed in cold acetone and incubation at room temperature for 20 minutes.

Mitochondrial enzymes

Succinic dehydrogenase was measured by the method of Pearse using 2,5-diphenyltetrazolium bromide (MTT) as the tetrazolium salt with incubation for 30 minutes at 37°C (Pearce 1960). Reduced nicotamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate dehydrogenase were measured by the method of Scarpelli, Hess and Pearce (1958) with incubation at 30 minutes and 37°C.

Lysosomal enzyme

Non-specific acid phosphatase was measured by the method of Burstone (1958) using naphthol AS-TR-phosphate as the substrate and

fast dark blue R as the diazonium salt, the sections being fixed in cold formalin with incubation at 37°C for 90 minutes. In addition, the method of Gomori (cited in Pearce 1960) using lead sulphide was used. All histochemical reactions were assessed by 2 observers (M.H. Floch and D.J.C. Shearman) and comparisons between different animals were made only on specimens stained simultaneously.

Triglyceride measurements

Samples of liver from iron deficient and control rats were subjected to triglyceride measurements. These were kindly made by Dr. Robert Scheig. The amount of triglyceride was expressed in μ mol per gram of wet weight, the normal rat liver containing 5-12 μ mol per gram of wet weight.

Human Studies

Seventeen subjects who were suffering from moderate to severe iron deficiency anaemia were studied, together with a variable number of control patients for the various tests that were employed. Three patients with anaemia secondary to anticonvulsant therapy were also investigated. The initial gastric secretion test was usually performed during routine clinical investigation but any additional tests were voluntary on the part of the patient. These tests often caused the patient considerable discomfort at a time when they were feeling unwell from the effects of anaemia. In fact, investigation of many more than seventeen patients was attempted but only this small number completed the full investigation.

The diagnosis of iron deficiency was based on full blood counts and a stained blood film but in the majority, confirmatory evidence was obtained in the form of the demonstration of a low serum iron level and the absence of stainable iron in the bone marrow. The presence or absence of clinical evidence of chronic iron deficiency such as atrophic glossitis or koilonychia was also recorded.

Gastric acid secretion by these patients was investigated by means of the augmented histamine secretion test (Kay 1953), the histamine infusion test (Lawrie, Smith and Forrest 1964) or by stimulation with pentagastrin (Multicentre Pilot Study 1967). The same batch of histamine was used for all the infusion tests. After an overnight fast, a radio-opaque nasogastric tube was passed into the body of the stomach and

was positioned under fluoroscopic control. The resting secretions were aspirated and a basal 30 or 60 minute collection was made. Fifty mg. of mepyramine malleate was administered prior to histamine injection / infusion. Histamine 0.4 mg/kg was the dose used in the secretion test. In the histamine infusion test, the constant intravenous infusion of histamine was given by means of a Braun infusion pump. Doses of histamine used in the infusion test were 0.04 mg/kg. body weight and 0.1 mg/kg. body weight, each dose being infused for two hours. With the patient lying on the left side continuous suction was applied to the gastric tube and successive ten minute samples(or fifteen minute samples when the volumes were small) were collected. Collection was found to be assisted considerably by frequent suction with a syringe,and by deep breathing on the part of the patient. Saliva was not swallowed by the patient. All gastric aspirations were supervised by a nurse specifically trained in the technique. The volume of each sample of aspirated gastric juice was measured and the concentration of hydrochloric acid determined by the titration with 0.1 N NaOH to a pH of 7 using a Beckman, Model 72, pH meter. The total output of hydrochloric acid in mEq per 10 or 15 minutes was then calculated as a product of the volume in mls. and the concentration in mEq/litre. In some patients, the pepsin content of the sample of gastric juice was determined by the method of Hunt (1948) as modified by Bitsch (1966). Measurement of gastric juice intrinsic factor was also made in some cases (See Section 2).

Whenever possible, a biopsy of the mucosa of the body of the stomach was taken with the Crosby capsule (Crosby and Kugler 1957) the capsule always being positioned under fluoroscopic control. Biopsy was

usually taken before the patient left the X-ray machine in order to avoid any movement of the Crosby capsule during movement of the patient's body. A preliminary study of the biopsy material was made with a dissecting microscope ; the interpretation of such material may be restricted by the size and orientation of the sample which it was sometimes necessary to re-embed. The embedding process was facilitated by controlling the manipulation with a stereoscopic microscope. Sections were stained with haemotoxylin and eosin and by the periodic acid-Schiff method. In reviewing gastric biopsies, the following categories were defined :-

Normal

The essential feature which contrasts with the categories listed below is that the lamina propria, which is very small in volume, contains only scanty lymphocytes and plasma cells.

Superficial gastritis

There is no loss of secreting cells but beneath the surface epithelium there is often a heavy infiltrate of lymphocytes and plasma cells. This does not extend to the deeper layers of the mucosa.

Atrophic gastritis

There is a decrease in the number of glands and specialised cells and the normal regular architecture of the glands is disorganised. There is infiltration with lymphocytes, plasma cells and macrophages. There may be intestinal metaplasia.

Gastric atrophy

There is complete loss of parietal and chief cells but there is

often less infiltrate and metaplasia is common.

Parietal cell antibodies were looked for in all patients by means of the Coons indirect fluorescent antibody technique (Irvine 1963). Only patients with definite positive reactions were actually recorded as positive. Intrinsic factor antibodies were looked for in all patients by the charcoal method (Ardeman and Chanarin 1963). To avoid any false positive reactions, care was taken to see that all serum obtained from patients for this measurement was taken at least 48 hours after any vitamin B_{12} injection. Sera recorded as positive gave values greater than 5 ng.units/ml. Vitamin B_{12} levels in the serum were determined by microbiological assay with Euglena gracilis, strain Z. The method was that of Anderson (1964) which makes use of a serum inhibitor in the standard. The range of normal by this method is 163 to 925 pg/ml. and the range for pernicious anaemia is 9 to 113 pg/ml. Serum folic acid was measured by the method of Waters and Mollin (1963). Vitamin B_{12} absorption was assessed whenever possible by the Schilling test, the flushing dose being given 2 hours after the oral dose of 0.5 μg (0.5 μc) of radioactive vitamin B_{12}. When this test showed a malabsorption of vitamin B_{12}, an attempt was made to correct the test by giving the patient an oral dose of intrinsic factor. Malabsorption was regarded as present when there was less than 7.5% of the oral dose excreted in the urine in 24 hours ; equivocal absorption 7.5 to 12.5% and normal absorption over 12.5%. The serum iron and iron binding capacity estimations were performed by the Clinical Chemistry Laboratory of the Royal Infirmary. Serum iron was measured by the method of

Young and Hicks (1965) and iron binding capacity by the method of Ramsay (1957). Normal values for iron are (males) 80 - 180 µg/100 ml., (females) 60 - 160 µg/100 ml. Total iron binding capacity 250 - 400 µg/100 ml.

RESULTS

IRON DEFICIENCY IN RATS

The weights and haemoglobin levels of the groups of rats in the histological and histochemical studies are shown in Table I. The iron deficient group resulted from the feeding of pregnant rats with the low iron diet from the 7th day of pregnancy to the final day of gestation, the diet being continued throughout the period of lactation and nursing. The infant rats were then fed on the same diet. The growth of the infant rats was greatly impaired, they appeared weak and listless and their coats were thin and sparse (Figs 1 and 2).

The weights and haemoglobin levels of 4 groups of rats which were used for gastric acid studies are shown in Table 2. These rats were killed at 42 days. Groups 1 and 2 which were prepared on an iron deficient diet given only after weaning showed a normal growth although they were moderately anaemic. Groups 3 and 4 showed impairment of growth although the haemoglobin levels attained were not as low in these rats whose mothers commenced the iron deficient diet at the 14th day of pregnancy, as when the diet was commenced on the 7th day of pregnancy. (Table 1).

Haematological Findings

The haemoglobin levels are listed in Tables 1 and 2. The blood films of Group 1, Table 1 and Groups 1 - 4, Table 2 showed severe hypochromia. The haemoglobin values for the mothers were normal at the time of birth but were reduced to an average of 9g% 20 days after

Table 1. Average weights and haemoglobin levels in three groups of rats used for histological and histochemical studies

	Number	Weight (g.)		Haemoglobin (g%)
		28 days	42 days	
Iron deficient rats	20	21	52	1.0 - 2.1
Age Control rats	10	122	147	16.0
Weight control rats	10	25 - 50		16.0

Figure 1. A 28 day old weanling rat with a haemoglobin level of 2g%. The rat shows poor growth and development and has a very sparse coat (X1).

Figure 2. A 42-day old iron deficient weanling rat alongside a normal rat (X0.3).

Table 2. Weight and haemoglobin ranges in 4 groups of iron deficient rats used for acid studies.

Experiment No.	No. of rats	Weight (g) range	Haemoglobin (g%) and average range	Preparation
1	13	124 - 174	7.9 (5.3-9.8)	Iron deficient diet given after weaning.
2	16	135 - 207	8.0 (5.6-9.7)	Iron deficient diet given after weaning.
3	13	35 - 68	3.6 (2.7-5.0)	Iron deficient diet from the 14th day of pregnancy.
4	15	50 - 102	5.0 (3.7-7.7)	Iron deficient diet from the 14th day of pregnancy.

parturition. The average haemoglobin levels in control mothers, weight controls (Table 1) and age controls (Table 1) was 16g%.

Histological and Histochemical Findings

Stomach. At both 28 and 42 days after birth there was a patchy decrease in the number of parietal cells and the thickness of the mucosa in some of the iron deficient animals but not in the age or weight control animals. In the areas of parietal cell decrease, there was no increase in lymphocytes or plasma cells. There was no change in the distribution of cytochrome oxidase or dehydrogenase activities. The only minor changes noted was an increase in acid phosphatase in some parietal cells.

Small Intestine. There were no significant histological differences between the iron deficient and the control rats. However, there was a definite increase in alkaline phosphatase and esterase activity and a less marked increase in acid phosphatase, succinic dehydrogenase, leucine aminopeptidase and reduced nicotinamide adenine dinucleotide. There was no change in the intensity of staining for cytochrome oxidase.

Colon. There were no histological changes, but minor histochemical changes as for small intestine were noted.

Liver. Macroscopically the liver was a pale yellowish pink colour in the severely iron deficient animals in contrast to the red colour of the normal rat liver. Histologically there was moderate to severe central liver cell damage, the hepatocytes in these areas were enlarged and numerous vacuoles were present in the cytoplasm. (Figs. 3,4 & 5).

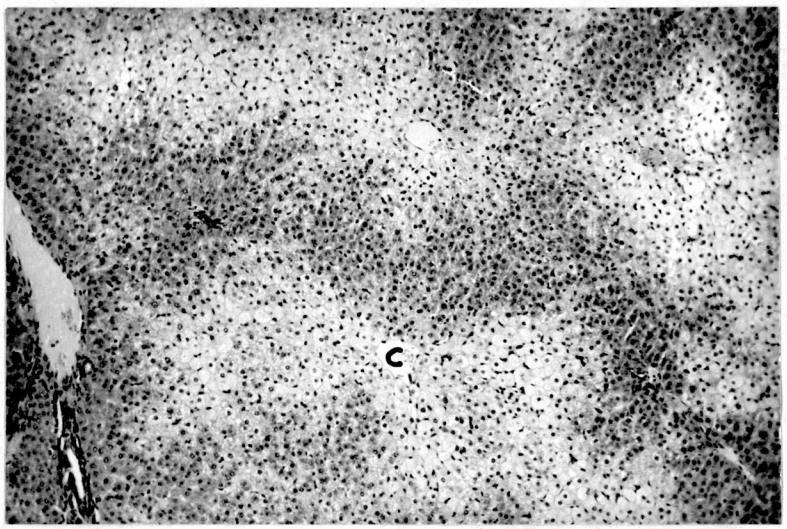

Figure 3. Histology of liver from 28 day iron deficient rat (haematoxylin and eosin).
The central vein (c) is seen surrounded by areas of necrosis (X130).

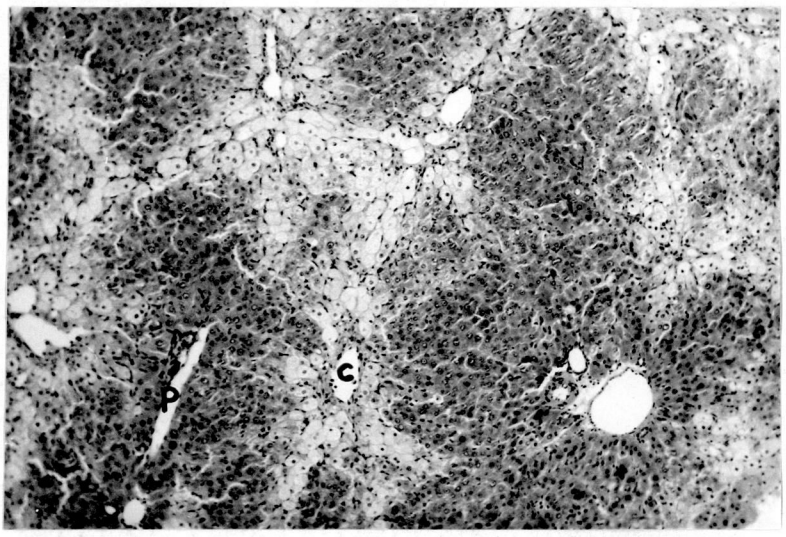

Figure 4. Histology of liver from 28 day iron deficient rat (haematoxylin and eosin).
The central vein (c) is surrounded by necrosis whereas the portal area (P) is surrounded by unaffected cells (X120).

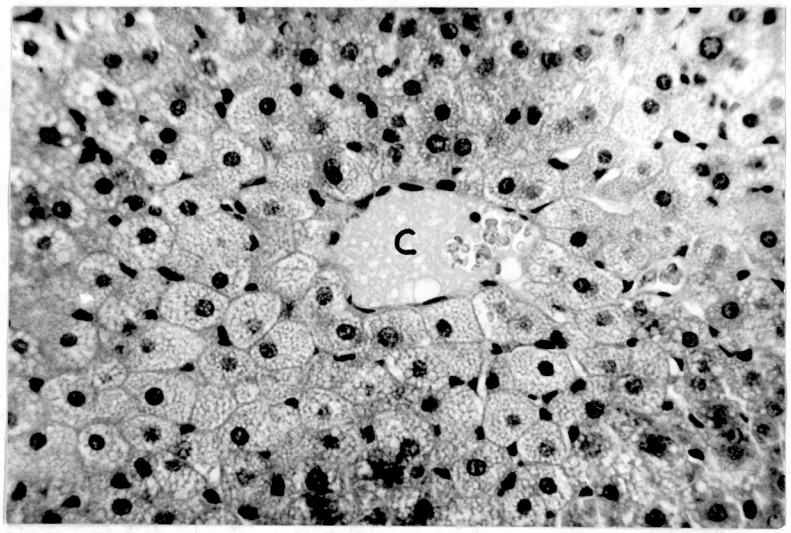

Figure 5. Histology of liver from 28 day iron deficient rat (haematoxylin and eosin).
The hepatocytes surrounding the central vein show necrosis and vacuolisation (X500).

These vacuoles stained positively for fat with Sudan black, flamingo red and oil red O stains (Figs. 6, 7 & 8) but they did not stain positively for glycogen. It will be seen (Fig. 8) that fat droplets were much larger in cryostat sections than in tissue fixed in formaldehyde solution stained with Sudan black.

In the sections stained for succinic dehydrogenase (Fig 9) there was a decrease in formazan granule deposition in hepatocytes in the hepatic central areas. Paradoxically, there was a slight increase in reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate. The periportal areas appeared normal. Both the cytochrome oxidase and non-specific esterase reactions showed normal localisation of activity with a suggestion of a generalised decrease throughout the tissue.

Triglyceride Measurements

There was 16μmol of triglyceride per gram of wet weight in the livers of iron deficient rats killed at 42 days in contrast to 5-12μ mol per gram wet weight in the livers of age and weight control animals.

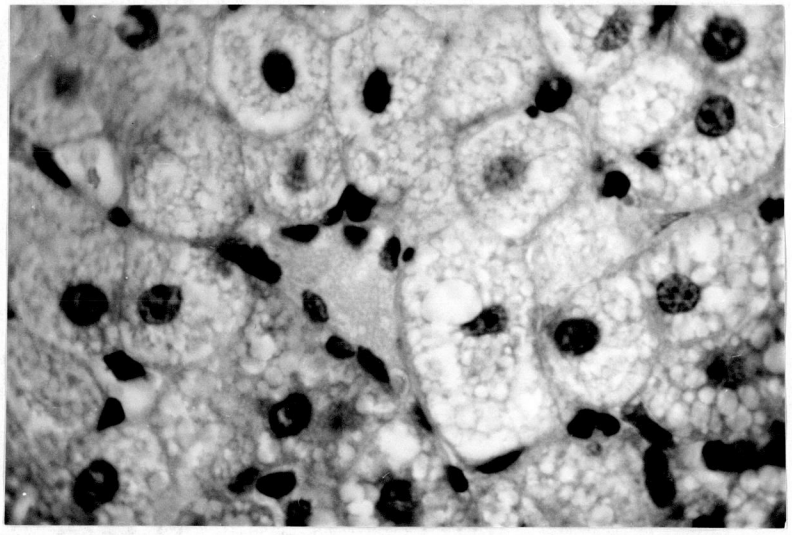

Figure 6. Liver from 28 day iron deficient rat.
Fat stains.
Hepatocytes from the central zone.
The vacuoles stain positively for fat
(Sudan black and flamingo red) X 825.

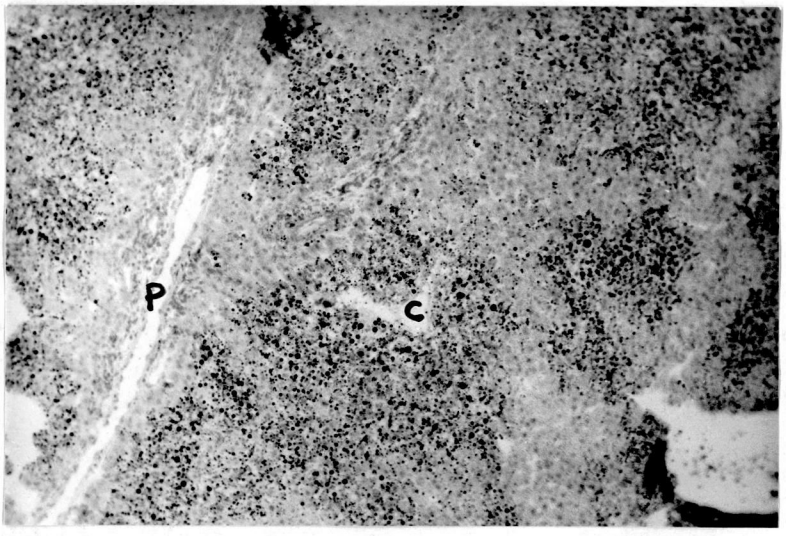

Figure 7. Liver from 28 day iron deficient rat. Fat stains. Hepatocytes with portal and central vein zones. The black areas represent fat which is concentrated around the central vein rather than around the portal zone. (Oil red O) X 100.

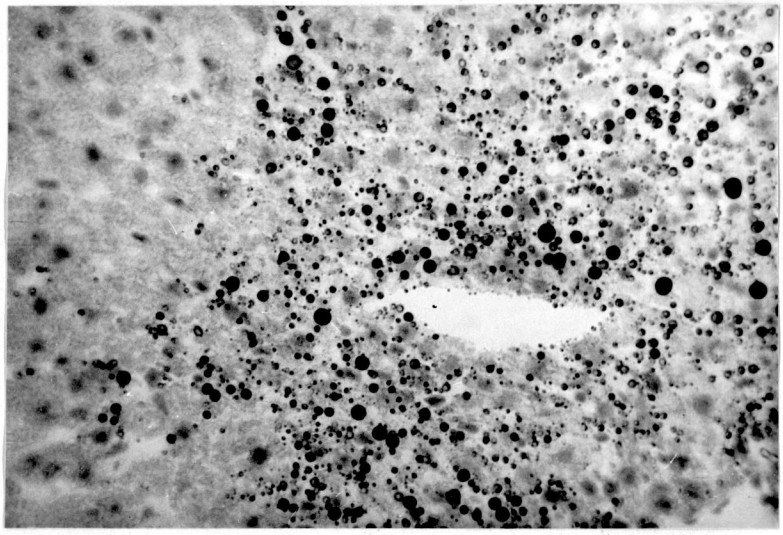

Figure 8. Liver from 28 day iron deficient rat.

Fat stains.

Higher power view of central zone.

Fat globules stain black (Oil red O) X 730.

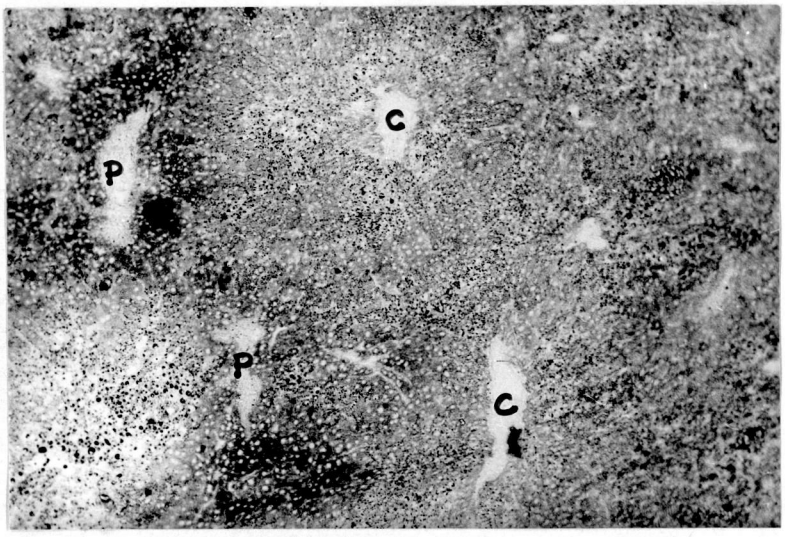

Figure 9. Liver from 28 day iron deficient rat. Succinic dehydrogenase reaction. The portal zone (P) is surrounded by a black homogeneous stain (formazan granules) which is in the hepatocytes. This is not present in the hepatocytes around the central vein (c) although some fat has been stained in these areas (X 72).

Gastric Acid Secretion Studies in Iron Deficient Rats

The results are summarised in Tables 3 - 6. The rats which received the iron deficient diet after weaning (Tables 3 and 4) were moderately anaemic but showed no growth impairment. They were capable of secreting acid normally, apart from those rats which had bleeding into the stomach after the operation. Two groups of rats (5 and 6) were severely anaemic. In these groups the total acid produced was less but it was compatible with the weight of the rat. In group 3 (Tables 2 and 5) which was the most anaemic tested, the pH of the gastric juice when undiluted was within the range pH 1.0 to pH 1.5.

Table 3. Experiment 1. - Gastric acid Secretion in Iron deficient rats

Rat No.	Volume of gastric juice (ml)	pH*	µEq Acid	Comments
1	1.6	2.2	126	
2	4.5	2.1	214	
3	3.2	7.0	0	Blood present
4	3.8	3.7	90	Blood present
5	3.0	2.0	233	
6	4.5	1.6	330	
7	2.7	2.5	93	
8	5.0	1.5	444	
9	3.0	2.6	144	
10	4.2	2.1	215	
11	2.1	2.1	168	
12	4.0	2.3	140	
13	3.0	7.0	0	Blood present

* pH recorded after volume made up to 10 ml. with distilled water.

Table 4. Experiment 2. Gastric acid Secretion in Iron deficient rats

Rat No.	Volume of gastric juice (ml)	pH*	µEq Acid	Comments
1	149	2.3	139	
2	146	1.8	318	
3	194	1.6	466	
4	207	1.5	519	
5	150	3.1	76	
6	162	1.7	311	
7	122	3.1	98	Blood present
8	163	2.3	211	
9	178	1.6	411	
10	198	2.5	115	
11	168	3.4	141	
12	168	2.2	241	Blood present
13	148	2.2	179	
14	135	2.7	142	Blood present
15	170	1.5	446	
16	144	2.6	110	

* pH recorded after volume made up to 10 ml. with distilled water.

Table 5. Experiment 3 - Gastric acid secretion in iron deficient rats

Rat No.	Volume of Gastric Juice (ml.)	pH*	µEq Acid	Comments
1	0.8	2.6	57	
2	2.0	2.4	111	
3	2.4	2.0	212	
4	1.5	2.2	123	
5	1.8	2.1	169	
6	0.6	2.5	51	
7	2.3	2.3	138	
8	1.0	2.6	76	
9	0.8	2.6	65	
10	Died			
11	0.4	3.6	15	
12	1.0	3.1	63	Blood present
13	Died			

* pH recorded after volume made up to 10 ml. of water.

Table 6. Experiment 4. Gastric acid Secretion in Iron deficient rats

Rat No.	Volume of gastric juice (ml)	pH*	µEq Acid
1	4.2	2.0	213
2	4.5	1.8	332
3	4.0	1.6	322
4	4.4	1.8	293
5	4.0	1.7	317
6	6.0	1.5	500
7	6.0	1.7	443
8	1.5	2.0	161
9	2.2	2.0	214
10	Died		
11	0.8	2.5	60
12	3.0	1.9	251
13	2.6	1.9	234
14	3.5	1.7	343
15	4.0	1.7	420

* pH recorded after volume made up to 10 ml. with water.

IRON DEFICIENCY, GASTRITIS AND GASTRIC FUNCTION IN THE HUMAN

Clinical details of patients with iron deficiency

The clinical details of 17 patients with chronic iron deficiency are listed in Tables 7 and 8. In each case, evidence of alimentary tract pathology which could cause bleeding was sought. Only after a barium meal examination, a dietary and menstrual history and the frequent examination of specimens of stool for faecal occult blood was a decision made as to whether the cause of the iron deficiency was menorrhagia, dietary inadequacy or whether it was due to alimentary blood loss. No actual measurement of menstrual blood loss was made. Patients in whom a source of blood loss could not be found are listed as idiopathic cases. Glossitis was more common in patients who secreted acid (Table 7) than in those who were achlorhydric (Table 8) Koilonychia was present in three patients in each group. The presence or absence of either clinical finding was not related to the degree of iron deficiency.

TABLE 7

Clinical and laboratory features of patients presenting with chronic iron deficiency anaemia who were not achlorhydric

Case No.	Sex	Hb(g%)	Glossitis	Koilony-chia	Serum iron (µg%)	Total iron binding capacity	Stainable marrow iron	Parietal cell anti-bodies	Vitamin B_{12} absorption (% radio-activity recovered in urine)	Gastric biopsy	Barium meal	Probable cause of iron deficiency	Serum vit. B_{12} (µµg/ml)
1	F	7.2	Present	Present	20	537		Negative		Not done	Not done	Menorrhagia	440
2	F	5.0	Present	Absent	18	474		Negative	15	Some atrophic gastritis	Normal	Idiopathic	-
3	M	2.5	Present	Absent	<10	381	Trace	Negative	11.4	Partial atrophy	Normal	Dietary	442
4	F	6.3	Present	Absent	46	344		Negative	15.7	Normal	Normal	Menorrhagia	227
5	F	4.1	Present	Present	28	546	Absent	Negative	-	Not done	Normal	Menorrhagia	551
6	F	7.9	Present	Absent	25	405	Absent	Negative	17.4	Some atrophic gastritis	Normal	Menorrhagia	552
9	F	8.5	Present	Absent	38	411		Negative	27.2	Not done	Not done	Menorrhagia	395
10	F	6.1	Present	Present	20	525	Absent	Negative	13.7	Not done	Normal	Idiopathic	571
11	F	5.1	Present	Absent	16	321		Negative	14.1	Some atrophic gastritis	Normal	Duodenal ulcer	240

Table 7.

TABLE 8

Clinical and laboratory features of subjects presenting with chronic iron deficiency anaemia and achlorhydria

Case No.	Sex	Hb(g%)	Glossitis	Koilony-chia	Serum iron (µg%)	Total iron binding capacity (µg%)	Stainable marrow iron	Parietal cell anti-bodies	Test for intrinsic factor antibodies	Vitamin B_{12} absorption (% radio-activity recovered in urine)	Gastric biopsy	Barium meal	Probable cause of iron deficiency	Serum Vit. B_{12} (µµg/ml)
7	F	3.8	Present	Present	32	444	Absent	Negative	Negative	13.1	Not done	Normal	Dietary	192
8	F	7.4	Absent	Absent	20	480		Positive	Negative	9.9	Chronic atrophic gastritis	Normal	Idiopathic	164
12	F	9.9	Absent	Present				Strongly positive	Negative	14.2	Not done	Normal	Idiopathic	-
13	F	7.6	Present	Absent				Positive	Negative	15.0	Chronic atrophic gastritis	Not done	Idiopathic	418
14	F	9.5	Absent	Absent				Positive	Negative	3.9	Chronic atrophic gastritis	Normal	Menorrhagia	88
15	F	9.1	Present	Present	40	522	Absent	Positive	Negative	2.0	Chronic atrophic gastritis	Normal	Idiopathic	89
16	M	6.7	Present	Absent	50	576	Absent	Positive	Negative	3.7	Chronic atrophic gastritis	Normal	Idiopathic	315
17	F	8.8	Absent	Absent	30	525	Absent	Strongly positive	Negative	11.5	Gastric atrophy	Normal	Idiopathic	361

Table 8.

Gastric Secretion of hydrochloric acid in iron deficiency anaemia

The gastric secretion of hydrochloric acid under augmented histamine stimulation in 17 patients is shown in Table 9. The corresponding levels of haemoglobin in these patients before and after treatment are also shown.

Eight of the 17 patients were achlorhydric and in the remaining patients the secretion of hydrochloric acid was depressed. It will be seen that in the majority of patients in whom acid was originally present in the stomach, there was some increase after iron therapy. The achlorhydric patients showed no such rise except in Case No. 13 where there was a secretion of 0.3 mEq/HCl in the post-histamine hour after iron therapy. The re-testing of patients was undertaken at least 2 to 3 months after their haemoglobin had returned to normal levels.

In an attempt to determine whether the rise in acid secretion after oral iron therapy was due to a repletion of the tissues with iron or whether it was due to the correction of the anaemia the following studies were undertaken:

One patient (Case No. 4) with a histamine response of 6.6 mEq/hr. was given 0.85g of saccharated iron oxide intravenously over a period of five days and the histamine infusion test was repeated on the fifth day. The test then gave a result of 6.9 mEq/hr. A second patient (Case No. 6) who produced 15.9 mEq/hr. was transfused with 7 units of packed cells over the period of 48 hours and the histamine infusion test repeated a few days later. She then produced 12.9 mEq/hr. Six

Table 9. Gastric secretion of hydrochloric acid in subjects suffering from chronic iron deficiency anaemia

Case No.	Sex	Hb.G%		Gastric Secretion (mEq.HCl/hr)		Test of gastric acid secretion
		Before treatment	After treatment	Before treatment	After treatment	
1	F	7.2	12.9	2.4	2.7	Histamine infusion test
2	F	5.0	14.6	10.1	11.5	-do-
3	M	2.5	13.7	4.8	15.9	-do-
4	F	6.3	13.2	6.6	11.3	-do-
5	F	4.1	-	15.4	-	-do-
6	F	7.9	14.6	15.9	18.5	-do-
7	F	3.8	13.6	0.0	-	-do-
8	F	7.4	14.6	0.0	0.0	-do-
9	F	8.5	13.6	11.9	17.3	Histamine secretion test
10	F	6.8	13.7	3.6	6.7	-do-
11	F	5.1	12.7	14.4	17.1	-do-
12	F	9.9	13.5	0.0	0.0	-do-
13	F	7.6	13.7	0.0	0.3	-do-
14	F	9.5	14.8	0.0	-	-do-
15	F	5.1	13.6	0.0	0.0	-do-
16	M	6.7	14.6	0.0	0.0	-do-
17	F	8.8	13.9	0.0	0.0	-do-

months after this therapy when both cases had a normal level of haemoglobin, Case No. 4 produced 11.3 mEq/hr. and Case No. 6 produced 18.5 mEq/hr. These results are summarised in Table 10.

Table 10. The effect of rapid therapy by parenteral iron or blood transfusion on gastric secretion in iron deficient patients

Case No.	Therapy	Before therapy		1 - 5 days after therapy		6 months after therapy	
		HCl (mEq/hr)	Hb (G%)	HCl (mEq/hr)	Hb (G%)	HCl (mEq/hr)	Hb (G%)
4	I.V. saccharrated iron oxide 0.85g	6.6	6.3	6.9	7.3	11.3	13.2
6	Blood transfusion Packed cells from 6 pints of blood	15.9	7.9	12.5	13.1	18.5	14.6

Gastric Secretion of acid in the anaemia of folic acid deficiency

In 3 patients, the gastric secretion of acid, pepsin and intrinsic factor was studied before and after correction of the anaemia. In these patients the anaemia was due to folic acid deficiency.

Patient 1 - The patient was a male, aged 59 at the time of diagnosis of the anaemia. For many years his epilepsy was treated with phenobarbitone 30 mg. t.i.d. and phenytoin 100 mg. t.i.d. At the time of diagnosis, Hb. was 4.6g% and the MCHC 32%. The marrow was megaloblastic with abundant iron and the serum vitamin B_{12} was at the lower limit of normal. The Schilling test showed 13% recovery. The serum folate was 1.9 mμg/ml. There was a full haematological response to folic acid. The improvement in acid and intrinsic factor output is shown in Table 11. The anticonvulsant treatment was continued throughout the period of testing.

Patient 2 - This patient was a male, aged 43 at the time of diagnosis of sideroblastic anaemia. The serum folate was 2.8 mμg/ml. He was treated with folic acid and pyridoxine and the Hb. at the time of the second gastric function test was 10.6g%. There was a marked increase in acid secretion after the partial correction of anaemia.

Patient 3 - This patient was a female aged 35 at the time of diagnosis of megaloblastic anaemia. For epilepsy, she had received phenobarbitone for 22 years and primidone for $1\frac{1}{2}$ years. The Hb. was 5.7g% and the marrow was frankly megaloblastic with ample iron. The serum iron was 300 μg/100 ml. The serum vitamin B_{12} level was 136 μμg/ml.

TABLE 11

Gastric function tests on 3 patients with anaemia and folic acid deficiency

	Patient 1				Patient 2		Patient 3	
	Before Treatment	After Treatment			Before Treatment	After Treatment	Before Treatment	After Treatment
Date	15.12.65	31.1.67	7.11.67	5.4.68	16.11.67	3.4.68	29.4.68	27.5.69
Stimulant	Histamine 0.04mg/kg	Histamine 0.04mg/kg	Pentagastrin 6 µg/kg	Histamine 0.04mg/kg	Pentagastrin 6 µg/kg	Pentagastrin 6 µg/kg	Pentagastrin 6 µg/kg	Pentagastrin 6 µg/kg
Volume of secretion in post-histamine or post-pentagastrin hour (ml.)	40	42	74	72	308	422	58	58
Minimum pH in any 10-minute sample	5.7	3.0	1.1	1.3	1.1	1.7	1.6	1.0
Total acid (m-equiv.)	0.09	0.5	3.6	2.7	19.4	31.6	0.6	3.3
Maximum concentration (m-equiv.)	5	28	88	48	76	92	20	92
Total intrinsic factor in post-histamine or post-pentagastrin hour (ng.)	-	2,280	-	9,660	38,200	29,900	-	-
Total pepsin in post-histamine or post-pentagastrin hour (mg.)	-	3.0	-	16.3	-	-	-	-

Table 11.

and the absorption of vitamin B_{12} was normal. The serum folate was 0.9 mμg/ml. The first gastric function test was performed two days after the commencement of oral folic acid, the Hb. being 10g%, the rise being due to the transfusion of packed cells. At the time of the second gastric function test, Hb. was 13.0g% and the folate was 6.0 mμg/ml. and the anticonvulsant therapy was unchanged.

All three patients show a rise in total acid output in the months after treatment (Table 11) and this is also reflected in the minimum pH in any 10 minute sample and the maximum concentration of acid in any one sample. In patient 1 there was a marked rise in pepsin and intrinsic factor output in the 10 months between tests 2 and 3.

Gastric acid responses to two doses of histamine in patients with iron deficiency anaemia

The clinical details and acid secretions of seven patients with iron deficiency anaemia and six control patients are listed in Tables 12 and 13. These patients were subjected to histamine infusion tests in which two differing doses of histamine were infused successively. Each figure for acid secretion in Tables 12 and 13 represents the number of mEq of acid secreted per hour once a steady secretory state had been obtained. It will be seen that in patients 1, 2 and 7 there was no increase in acid secretion with the increased dose of histamine. In cases 3 to 6, there was an average increase of 40%. In the control patients there was no such increase. Indeed, in control cases 4, 5 and 6 (Table 13), it is of note that the output of acid actually fell during the fourth hour of histamine infusion. In two iron deficient patients, gastric biopsy was not performed. In Case No. 4 it was normal and in Case No. 7 there were minimal changes.

Figure 10 (Case No. 4) illustrates the histamine infusion test of one of the patients who reached the steady secretory state at 0.1 mg/kg. Figure 11 (Case No. 6) shows repeat infusion tests on one patient. Both tests on this patient produced the same total amount of acid in response to the same total dose of histamine. In the first test, the 0.1 mg/kg. dose of histamine was infused first and it will be seen that there was a small decrease in acid output when the dose was decreased to 0.04 mg/kg. The second test was performed some days later when the patient had a normal haemoglobin level after transfusion. Here again,

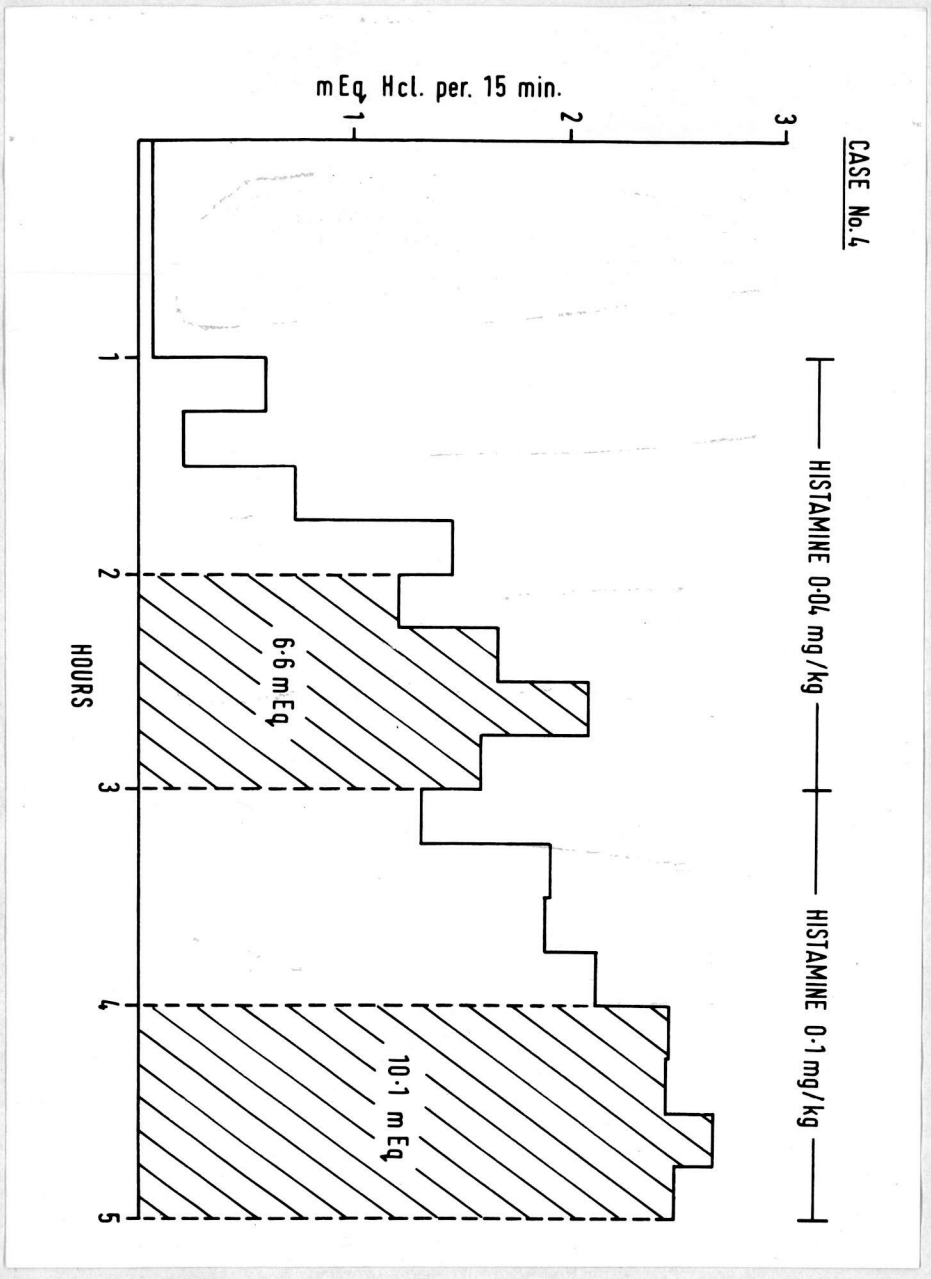

Figure 10. Histamine infusion test performed on Case 4.

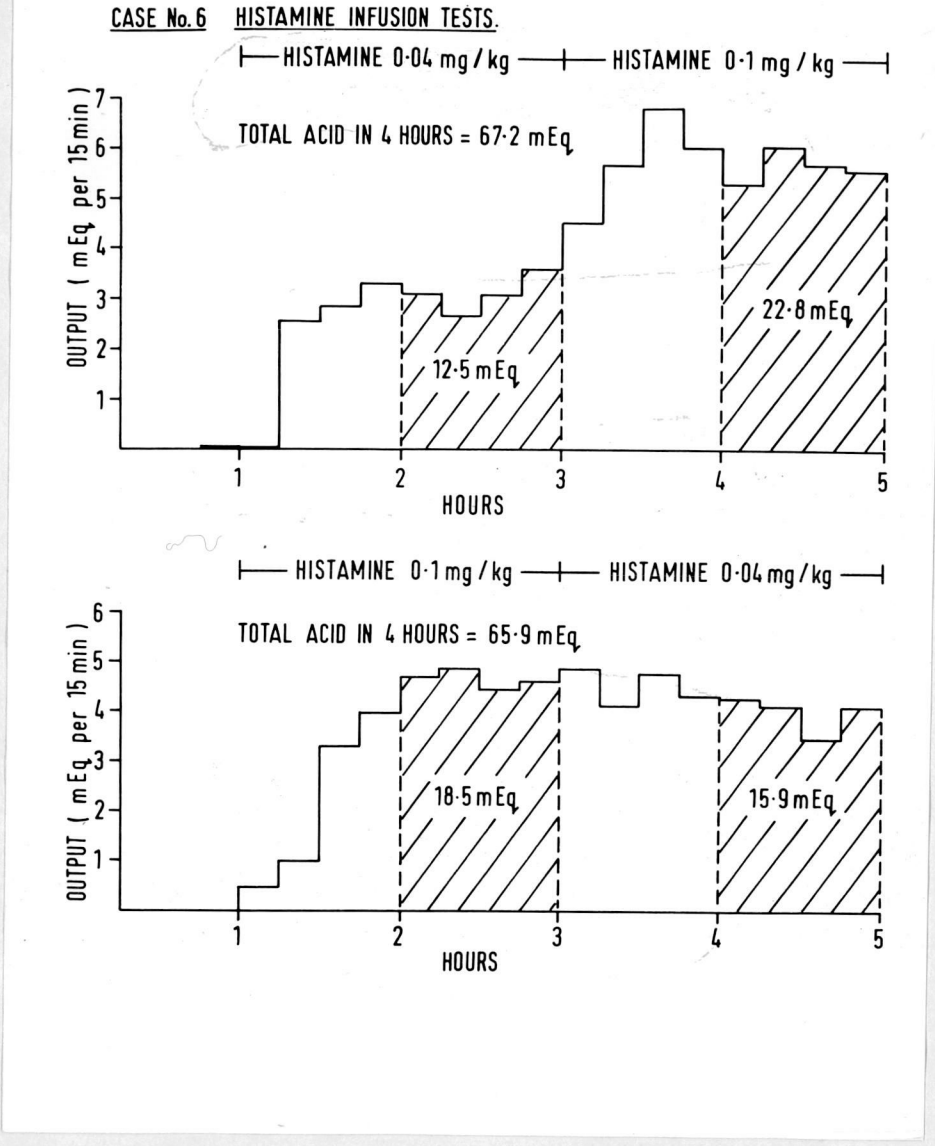

Figure 11. Histamine infusion tests performed on Case 6.

a difference in response to the two doses is seen, but the response to 0.04 mg/kg. is smaller and that to 0.1 mg/kg. is greater than before.

Gastric secretion of pepsin

Table 14 illustrates the pepsin determinations undertaken on patients with iron deficiency anaemia. It will be seen, as would be expected, that the pepsin levels with the two different doses of histamine correspond to the acid levels. This emphasises the difference in response with the two doses of histamine. In patient 7, who had acid responses of 15.8 and 15.9 mEq/hr, the pepsin response showed an increase with the 0.1 mg/kg. dose of histamine. This patient produced bile in the aspirate during the 0.1 mg/kg. histamine dosage, and this may have masked a rise in acid secretion.

Antibody studies : Vitamin B_{12} status

The patients have been divided into acid secretors and non-acid secretors and these two groups are the two groups presented in Tables 7 and 8.

Eight of the 17 cases presenting with iron deficiency anaemia were totally achlorhydric. Parietal cell antibodies were demonstrated in 7 out of 8 achlorhydric patients but not in any of the patients who secreted acid. None of these eight achlorhydric patients had antibodies to intrinsic factor. On the other hand, three had an abnormal Schilling test which was corrected by intrinsic factor and a further two patients had an equivocal absorption of radioactive B_{12}. Two patients had a low concentration of vitamin B_{12} in the serum. Patients 15 and 17 had a

Table 14. Acid and pepsin levels in 4 patients with iron deficiency anaemia.

Case No.	Acid (mEq/hr)		Pepsin (mg/hr)	
	0.04 mg/kg	0.1 mg/kg	0.04 mg/kg	0.1 mg/kg
4	6.6	10.1	47.5	64.4
5	15.4	18.1	86.7	92.4
6	12.5	22.8	44.4	73.3
7	15.8	15.9	39.1	45.6

strong family history of pernicious anaemia but they themselves presented as cases of iron deficiency anaemia and not as pernicious anaemia.

Gastric biopsy

Gastric biopsy was carried out before treatment in 11 patients. Only when the plane of the histological section included the whole thickness of the mucosa was the diagnosis of atrophy attempted. Lymphocytes and plasma cells are normally present in the gastric mucosa and gastritis was identified only when the criteria listed earlier were fulfilled.

In the achlorhydric patients, there was an absence of parietal cells and the presence of chronic atrophic gastritis was confirmed. This gastritis was often severe with an increase in the number of plasma cells and lymphocytes.

Five patients in whom acid was present had biopsies taken. Case No. 2 showed partial glandular atrophy with round cell infiltration. Case No. 3 showed chronic atrophic gastritis of moderate degree and there was a cellular infiltration with round cells and polymorphs. Cases 6 and 11 showed some atrophic gastritis, both these cases having some preservation of chief and parietal cells. Case No. 4 was histologically normal.

DISCUSSION

Methods of inducing iron deficiency in the rat

In previous experiments to test the effects of iron deficiency on the mucosa and secretions of the gastrointestinal tract, anaemia has been induced by feeding a low iron diet or by bleeding the animals or by a combination of these two methods. Although the rat has very small body stores of iron, iron deficient diets have not produced gastrointestinal tract lesions and this failure may be due either to insufficient dietary restriction of iron or to the short period of study. In some cases (Valberg, Taylor, Witts and Richardson 1961), Binder, Fischer, Thayer, Spencer and Spiro 1966) both the methods were probably inadequate. In rat and man, iron balance is most precarious at the end of the weaning period at a time when there are no exogenous sources of iron. This is a period of rapid growth when the iron stores are quickly utilised and the milk is a very poor source of iron (Blaxter 1961). In the present study, iron deficiency was induced in pregnant rats at a stage when their small body stores would be contributing to the offspring in utero. It was hoped that severe anaemia would be induced in the offspring and this would cause an impairment of the build up of iron containing enzymes in the tissues. The method was found to produce a severe anaemia in the infant rat but many animals were so weak that they died after 40 days or even before. The profound effect on growth was limiting to experiments since many animals could not withstand anaesthetics and operations. The effects of the anaemia on the tissue enzymes in these animals will be discussed

later. The principle of this method has been used by Beutler (1959) and Dallman (1969) but in these latter experiments, the iron chelating agent desferrioxamine was used to treat the pregnant and nursing mother rats. Murray and Stein (1968) attempted to induce iron deficiency by feeding undiluted evaporated cow's milk (Carnation) with copper supplements. At 6 months, the rats were not anaemic and growth and nutrition were normal. They were then bled from the tail at a rate of 4 ml weekly for 7 weeks at which time they were anaemic and chemical analysis of the livers showed low iron stores. These rats were then compared with a control group of rats of the same weight which were anaemic but had normal iron stores. Such anaemia was induced by giving oestrogen (Murray and Stein 1968).

The Histology and Histochemistry of Alimentary Tract Mucosa in Iron Deficiency

Two factors that must be considered in the assessment of the varied findings by different workers are firstly the cellular turnover of the tissue in question and secondly the variable effect of iron deficiency on different iron containing enzymes. For example, using dietary measures alone, Dallman (1969) showed that in young rats, the depression of cytochrome c in intestinal mucosa was equal to or was more severe than the mean decrease of 34% in haemoglobin concentration. A more severe anaemia induced by giving an iron chelating agent to the mother also reduced growth rate but there was no greater effect on cytochrome c. It is of interest that in the human some enzymes, for example cytochrome oxidase (Dagg, Jackson, Curry and Goldberg 1966) may be

depleted in sideropenia without anaemia. On the other hand, in severe iron deficiency other enzymes would seem to behave in an 'inviolate' fashion (Beutler 1964). Cellular turnover in the mucosa of the gastro-intestinal tract can vary considerably. In the intestine, however, complete mucosal epithelial cell turnover varies from 24 hours in the upper small intestine to 72 hours in the colon. Dallman and Schwartz (1965) showed that the content of iron containing enzymes depended on turnover, In their iron deficient rats, cytochrome oxidase was diminished in the villous epithelial cells, but after iron had been given, increased staining appeared rapidly in the new cells arising from the crypts. This raises the possibility that for the intestinal mucosa, the iron containing enzymes in any one cell may be 'inviolate'. If this principle applied also to gastric mucosa and to the parietal cell in particular, the iron deficiency might need to be prolonged before changes could be induced because of the turnover rate of the parietal cell, which is thought to be at least 60 days (Lipkin, Sherlock, and Bell 1963 ; Macdonald, Trier and Everett 1964). Thus if a parietal cell had insufficient available iron to become replete with iron containing enzymes the induction of cellular iron deficiency would depend only on turnover time.

In the present studies, there were no consistent histological changes in the gastric and intestinal mucosae but there were some minor histochemical changes. (Shearman, Floch, Herskovic, Levine and Spiro (1967). In assessing these, it should be remembered that histochemical techniques are limited in their ability to demonstrate small differences

in the concentration of an enzyme and only major qualitative differences can be regarded as significant. Histologically, there was a minor patchy decrease in parietal cells in the severely iron deficient rats killed at 28 and 42 days, but there was no increase in the number of lymphocytes and plasma cells and therefore no features of gastritis. In addition, some parietal cells showed a decrease in cytochrome oxidase and dehydrogenase activity. From this it must be assumed that the iron deficiency in utero was insufficient to prevent formation of iron containing enzymes in the gastric mucosa, and after birth the animals did not live long enough in the iron deficient state to allow for the formation of iron depleted parietal cells. The presence of a few parietal cells depleted in enzymes at 42 days would suggest that this was occurring. The significance of the increase in acid phosphatase noted in some sections is not clear but it could be related to an increase in macrophages which were also noted. Since the completion of these studies on the rat, Stone (1968) has studied cytochrome oxidase activity in gastric biopsies from iron deficient human subjects. It was found that the activity of this enzyme was strong in all parietal cells and the total amount of activity was proportional to the number of parietal cells present in the sections.

The histochemical studies performed on intestinal epithelium showed a generalised decrease in enzymatic activity in the iron deficient rats, killed at 28 and 42 days. This is in-keeping with the studies by Dallman (1969).

Acid studies on iron deficient rats

In the human, achlorhydria and hypochlorhydria are common in iron deficiency and the controversy surrounding this association is discussed fully in the section on human iron deficiency. Relatively few acid studies have been performed on the iron deficient rat. Valberg, Taylor, Witts and Richardson (1961) showed that there was a reduction in gastric acid secretion with iron deficiency anaemia but as in studies in the human, it was not shown by these workers that the reduction was due to the anaemia itself.

In the present studies, severe anaemia was induced in some rats e.g. Table 1, Hb. 1.0 - 2.1g%. Unfortunately , in the anaemic rats used for the gastric acid studies, the anaemia was not so severe e.g. Table 2 Experiment No. 3 Hb. 2.7 - 5.0g%. There was no apparent impairment of gastric acid secretion as tested by the Shay technique (Shay, Sun and Gruenstein 1954). The study can be criticised because the groups of rats are not comparable in weight or haemoglobin level and weight controls were not used. However, this study was limited in time because it was performed in another department and it was designed to be a qualitative one which would define appropriate investigations when larger groups of rats could be prepared.

In experiment 3, (Tables 2 and 5) this group being the most anaemic of the four groups tested, all the rats were able to produce a gastric pH in the range pH 1.0 - 1.5, the pH results recorded in Table 5 having been made after bringing the volume of gastric juice to 10 ml with distilled water. Thus there was no loss of ability

to produce a very low pH. It would be possible, however, for there to have been a reduction in the total amount of acid secreted but Murray and Stein (1968) in studies discussed more fully below, found that when there was a reduced total secretion of acid then this resulted in a higher pH than in control groups.

In assessing the present results, it must be concluded that there was no marked impairment of acid secretion. The reasons for this are likely to be that the duration of severe iron deficiency (42 days) was short in comparison to the long turnover time of the parietal cell. The findings of normal acid secretion correlate well with the minimal histochemical changes found in the gastric mucosa of similar groups of rats, Presumably the parietal cells being tested acquired an adequate supply of iron containing enzymes while the rat was in utero. These conclusions tend to be supported by the more recent work of Murray and Stein (1968). They found a significant reduction in the total acid secreted and its concentration and a rise in gastric pH in iron deficient rats tested by the Shay technique. These rats were prepared over a much longer period of time in that they were bled weekly after a period of six months on a low iron diet. A control group of rats rendered anaemic by feeding oestrogen but which were not iron deficient, had a normal acid secretion. Thus moderate anaemia in the rat does not in itself cause a reduction in acid secretion.

Hepatic changes in iron deficient anaemic rats

In histochemical studies on the alimentary tract of the iron deficient rat, the liver has attracted little attention. In the present study, rats in the iron deficient group (Table 1) and those in Experiment 3 (Table 2) had livers which appeared macroscopically abnormal. Histological, histochemical and chemical studies confirmed that there was an increase in fat in these livers. The presence of intracellular fat was mainly in the central zones, the portal areas being relatively normal. There was a loss of succinic dehydrogenase in the central zones which could be interpreted as due to loss of iron stores in the main storage organ for iron. However, when the liver is injured by various means, the areas of necrosis show a decreased succinic dehydrogenase activity (Smith and Coote 1963). In the present study both reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphase were increased in activity. These are non-iron containing mitrochondrial enzymes and their increase suggests early mitochondrial disruption with enzyme release.

These findings are in favour of the liver lesions being caused by severe anaemia and resultant anoxia rather than by iron deficiency itself. The situation of the major changes in the central zones rather than in the portal areas would also be in favour of this.

The relationship of pernicious anaemia and iron deficiency anaemia

An association between these disorders has been claimed by many workers, for example Faber and Gram (1924), Maclauchlan and Kline, (1926), Heath (1932), Witts (1930) and Kauffmann and Thiessen (1924). In a more recent study on 371 patients with iron deficiency anaemia, there was a 5.4% incidence of pernicious anaemia (Beveridge, Bannerman, Evanson and Witts (1965).

In the present series, the presence of parietal cell antibodies in 7 out of 8 achlorhydric patients and the abnormalities of vitamin B_{12} absorption in some patients both agree with the postulated relationship between iron deficiency anaemia in the achlorhydric patient and pernicious anaemia (Shearman, Delamore and Gardner 1966). With regard to parietal cell antibodies, it should be noted that the sera of more than 90% of patients with pernicious anaemia contain these antibodies. It should also be noted, however, that parietal cell antibodies are quite commonly found in several other conditions besides pernicious anaemia and iron deficiency anaemia e.g. they are found in autoimmune thyroiditis (Ardeman and Chanarin 1963) thyrotoxicosis (Doniach, Roitt and Taylor 1963) and diabetes mellitis (Moore and Neilson 1963), and they also occur in a proportion of apparently healthy people. (See Section 2). It has been shown by Wright, Whitehead, Wangel, Salem and Schiller (1966) that there is a relationship between the presence of parietal cell antibody and the accompanying degree of chronic gastritis as demonstrated by gastric biopsy. None of the eight achlorhydric patients studied had antibodies to intrinsic factor. In

studies by various workers, antibodies to intrinsic factor have been found in patients with pernicious anaemia or with latent pernicious anaemia.

Thus it appears that the state of achlorhydria itself or possibly some other genetically determined disorder in the gastric mucosa results in an impaired absorption of iron. At a later stage, this situation may progress in some patients to inadequate production of intrinsic factor for normal absorption of vitamin B_{12}, but in the others, there may be no progression and the only markers of this state are the achlorhydria and the presence of parietal cell antibodies in the serum. Goldberg, Lochead and Dagg (1963) concluded that such a primary mechanism (an autoimmune process) was responsible for the production of gastric atrophy of a degree insufficient to cause pernicious anaemia but the changes induced must be sufficient to provoke the development or iron deficiency anaemia. For this to occur, it must be demonstrated that the absorption of iron in achlorhydric states is less than the absorption in patients with acid secreting stomachs. With regard to this evidence, there is a high incidence of iron deficiency anaemia in treated pernicious anaemia patients who have no obvious source of blood loss (Gibson, Kelly and Wang 1963) and patients with achlorhydria have a reduced iron absorption (Williams 1959, Goldberg, Lochead and Dagg 1963, Jacobs, Lawrie, Entwistle and Campbell 1966) However, it should be pointed out that Callender (1965) found no evidence of iron deficiency in patients suffering from pernicious anaemia and other studies have failed to show a reduced absorption of

iron in achlorhydria (Pirzio-Biroli, Bothwell and Finch 1958, Moore 1955, Biggs, Bannerman and Callender 1961).

It has been suggested that iron deficiency may cause an auto-immune reaction (Moulton 1964) or that gastric mucosal damage due to iron deficiency may cause the production of antibodies (McFadyen, Goldberg, Dagg and Anderson 1965) with consequent gastritis and depressed acid secretion. In this connection, it is of interest to contrast the presence of parietal cell antibodies in the achlorhydric iron deficient British subject with their absence in patients of Indian origin who were suffering from extremely severe iron deficiency anaemia due to hookworm infestation (Delamore, unpublished observations). This may be associated with the fact that Addisonian pernicious anaemia is thought to be rare in India. The present study, the results of which are summarised in Table 15 would seem to point to the fact that antibodies are not a secondary phenomenon and that in those cases in which they occur they are a reflection of the processes which lead to achlorhydria which in turn leads to malabsorption of iron and iron deficiency anaemia. Two recent papers would seem to confirm this relationship. Laws, Mollin and Coghill (1966) have shown that cases of atrophic gastritis many of which were associated with iron deficiency anaemia, had concomitant achlorhydria and parietal cell antibodies and Wright, Whitehead, Wangel, Salem and Schiller (1966) showed that 5 out of 9 patients with idiopathic iron deficiency anaemia had gastric parietal cell antibodies in their serum compared with only 2 patients out of 18 patients with atrophic gastritis who were suffering from a miscellaneous group of alimentary disorders, They conclude that the high prevalence of gastric

Table 15. Summary of clinical and laboratory findings on 17 patients with chronic iron deficiency.

	Patients with acid (9)	Achlorhydric patients (8)
Parietal cell antibodies	Absent	Present in 7
Gastric biopsy	Some degree of atrophic gastritis	Chronic atrophic gastritis in all patients biopsied.
Schilling Test	Normal	Abnormal in 5
Serum B_{12}	Normal	Low in 2
Cause of iron deficiency	Unknown in 2	Unknown in 6

gastric parietal cell antibodies in patients with gastritis and idiopathic iron deficiency anaemia as compared to patients who have an iron deficiency due to gastrointestinal bleeding, confirms the prediction (Delamore and Shearman 1965) that the mechanism of the gastritis in these two groups of patients might prove to be different.

Gastric Production of acid

The present findings confirm that there is a high incidence of achlorhydria and hypochlorhydria in patients suffering from iron deficiency anaemia. Such results have been made known previously by various workers. (Davidson and Markson 1955, Badenoch, Evans and Richards 1957, Bock, Richards and Witts 1963, Dagg, Goldberg, Anderson, Beck and Gray 1964). In those patients who underwent gastric biopsy the high incidence of achlorhydria and hypochlorhydria was associated with chronic atrophic gastritis. In the present group of 17 patients there are probably more achlorhydric patients than would be expected in any group of iron deficient individuals. However, no attempt at selection was made and the 17 patients represent 17 consecutive patients who presented with iron deficiency who were willing to undergo and complete the investigations.

Increase of acid secretion after therapy

There is an increase in acid production following therapy provided that the patient is not achlorhydric (Table 9). One patient did secrete 0.3 mEq. after treatment. It must be assumed that this small amount was missed in the initial histamine test. In these studies, the

histamine infusion test was used if possible because it is a more precise test in that the achievement of a steady secretory state indicates that the maximum rate of secretion has been reached. At the present time, it is impossible to say what brings about an increase of acid production in achlorhydric patients following iron therapy. It is now almost certain that the traditional concept that enzymes which contain iron are not affected in iron deficiency anaemia no matter how severe, is no longer acceptable. (Beutler 1964). Instead, apparently once the iron stores are depleted there is competition for the available iron between enzymes and other iron containing compounds, and haemoglobin. Some enzymes, e.g. cytochrome C and aconitase, seem to be depleted easily at least in certain tissues (Jacobs 1961, Beutler 1959, Beutler 1960), while others, e.g. catalase, seem to behave in an inviolate fashion (Beutler 1964). It is thus possible that treatment with iron could correct a functional deficit existing within the parietal cell in the state of iron deficiency. Another possibility is that correction of the anaemia itself restores, at least to some extent, the normal function of the gastric mucosa. Attempts to determine whether iron or haemoglobin deficiency were the prime cause of the hypochlorhydria did not produce a conclusive answer (Table 10). Treatment with intravenous saccharated iron oxide and transfusion with blood produced no increase in acid secretion in the two patients investigated by the histamine infusion test, although in one of these cases it was shown with time and the correction of the haemoglobin level, such an increase in the acid did actually occur.

It is probable that improved secretion is dependent upon the production of new parietal cells, the cellular turnover of which is thought to be slow both in the rat and in the human. Finally, it is possible that improved acid secretion following therapy may be associated with some other factor which is as yet poorly understood. For example, Bock and Witts (1963), showed a considerable increase in acid secretion following the treatment of thyrotoxic patients. It is of interest to note that of seven patients with tropical sprue treated by Vaish, Sampathkumar, Jacob and Baker (1965), six failed to increase their maximal acid output after treatment of the anaemia, but in one case there was a marked improvement in acid secretion coincident with the correction of the megaloblastic anaemia and an improvement in the patient's general condition as shown by a decrease in steatorrhoea and an increase in body weight. The studies on 3 patients with anaemia which was not due to iron deficiency, tends to confirm that other factors may play a part in the increased acid secretion after correction of anaemia (Shearman and Finlayson 1968). In these patients the same anticonvulsant treatment was continued throughout the studies. The improvement in secretion could have been due to the correction of folate deficiency or correction of anaemia. Certainly, in megaloblastic anaemia due to vitamin B_{12} deficiency, there may be cellular dysfunction in the intestinal cells as reflected in a malabsorption syndrome which is corrected by treatment with vitamin B_{12} (Herbert, Carmel and Li 1967). A similar cellular dysfunction might occur in the parietal cell in folate or vitamin B_{12} deficiency.

Stimulation of the gastric mucosa by two different doses of histamine

As will be seen from Table 12, some iron deficient patients showed an increase in acid production when they were stimulated with a 0.1 mg/kg. dose of histamine in contrast to the 0.04 mg/kg. dose. Figures 10 and 11 illustrate these points in two cases (4 and 6) who underwent a histamine infusion test. These results do suggest that the conventional maximal dosage of histamine of 0.04 mg/kg. body weight is not a maximal dose for some anaemic patients. In 4 out of 7 cases with iron deficiency anaemia it is shown that an increase in acid production can occur if the dose of histamine is increased. At the present time it is not known why some anaemic patients respond in this way whilst others do not. As previously mentioned, such an increase in acid output can be demonstrated more easily by the infusion test than by the augmented histamine test because the maximal response coincides with a steady secretory state. The inability of some anaemic patients to respond fully to conventional doses of histamine may be partly responsible for the increase in acid production after treatment discussed earlier and it may be a further reflection of a functional deficit of the parietal cell in iron deficiency anaemia. There are other conditions in which there is a variation in the sensitivity of the parietal cell to stimuli and in duodenal ulcer for example, the cell is more sensitive to gastrin (Mason and Giles 1968) in contrast to some patients with iron deficiency who appear to be relatively insensitive.

The responses of Case No. 6 are interesting because the differences

in response still existed after treatment of anaemia by blood transfusion. Although after treatment, the response to 0.1 mg/kg. was greater, the response to 0.04 mg/kg. was smaller than before. This could be because a large dose of histamine is required to initiate acid secretion by all the parietal cells of the stomach mucosa. It will be seen from Table 12 that in those patients with iron deficiency anaemia who were subjected to the two doses of histamine there was no correlation between the degree of anaemia, the gastric biopsy findings and the response to the stimulants.

Clinical evaluation of the iron deficient patient

The present studies resulted in the idea that patients with iron deficiency anaemia can be divided into two groups (Table 16) (Delamore and Shearman 1965).

Group I - This includes those patients in whom there has been chronic blood loss or deficiency of dietary iron intake severe enough to account for the anaemia and in whom there are no other causes for the development of chronic gastritis or gastric atrophy. In particular, these patients do not exhibit antibodies to gastric parietal cells.

Group II - This includes those patients who present with iron deficiency anaemia but in whom the reason for the deficiency is less obvious, that is, they are often termed idiopathic, and in whom there are other reasons why chronic gastritis might be present. The most important reason is the presence of gastric parietal cell antibodies, but it is possible that the patient could have malabsorptive disease, for example, tropical sprue, idiopathic steatorrhoea or coeliac disease. In all these patients, mucosal changes precede the onset of iron deficiency anaemia.

In patients of Group I, a deficiency of iron in some way results in the functional impairment of cellular activity in the gastric mucosa. At the moment, it is not known whether a deficiency of enzymes which contain iron or some other biological substance that requires iron, is responsible for the gradual atrophy of the gastric mucosa but if the

Table 16. Summary of theoretical findings in iron deficient patients.

Finding	Group 1	Group 2
Chronic blood loss or deficient iron intake	Present	Absent
Parietal cell antibodies	Absent	Somtimes present
Depressed acid secretion	Present	Present
Achlorhydria	Sometimes present	Usually present
Mucosal changes	Present	Present
Reversibility of depressed acid secretion	Present	Absent
Reversibility of achlorhydria	Absent	Absent
Reversibility of mucosal changes	Absent	Absent

process continues for a long period and the anaemia is very severe, complete atrophy could develop and be accompanied by achlorhydria. Before this stage is reached, some hydrochloric acid is secreted by the remaining parietal cells but this secretion may be relatively inefficient because the parietal cells are less sensitive to stimuli. Under 'maximal' histamine stimulation, some acid will apparently be produced if parietal cells are present even when the patient is severely iron deficient. The demonstration of achlorhydria by modern techniques indicates that all the parietal cells have been destroyed and that no return of function is to be expected to follow iron therapy.

In Group II patients, the gastritis probably precedes the anaemia and may result from a genetically determined disorder of immune tolerance or it can result from the extension of a disease process from elsewhere in the alimentary tract, for example, as in idiopathic steatorrhoea. These patients with parietal cell antibodies are usually achlorhydric ; they may have relatives who have pernicious anaemia. It is possible that they themselves may go on to develop pernicious anaemia but evidence upon this point is still lacking although some family studies have pointed in this direction (McFadyen, Goldberg, Dagg and Anderson 1967). It is perhaps more likely that they are cases of an immune disorder which is sufficient to cause achlorhydria and thus iron deficiency, but insufficient in intensity to cause complete destruction of intrinsic factor production by the gastric mucosa and so pernicious anaemia does not occur. Evidence to support this latter

view lies in the fact that intrinsic factor antibodies were not found in any of the patients in this series, nor in similar patients studied by other workers (Laws, Mollin and Coghill 1966, Wright, Whitehead, Wangel, Salem and Schiller 1966).

The suggestion that there are two groups of patients with iron deficiency anaemia associated with gastritis is not entirely new. As long ago as 1954 Leonard postulated that there was one group in whom the gastric lesion was congenital and possibly familial and in whom treatment did not restore acid secretion and another group in whom the defect might be secondary to iron deficiency. Some of his other suggestions have to be modified in the light of our knowledge today, but the basic concept is remarkably similar to the present hypothesis. Moreover, in 1960 Coghill stated that atrophic gastritis was more common in patients with iron deficiency anaemia in whom there was no evidence of blood loss or of exceptionally deficient iron intake. Apparently such patients would fit into Group II, and the idiopathic gastritis, which they were thought to have, represents a disorder of immune tolerance. It is likely that these are patients in whom gastric parietal cell antibodies are to be found.

Clinically, it may be useful to divide iron deficient patients into two groups but it is possible that some overlap may occur. If, for example, a patient with primary gastritis (consequent on a disturbance of immune tolerance) developed iron deficiency, from whatever cause, of such severity as to deplete the tissues of essential iron compounds, then a functional impairment might be added to the existing

structural impairment. In this event, therapy might bring about a small increase in acid secretion at least temporarily. On the other hand, if the chronic deficiency of iron in patients in Group I is of sufficient severity and duration to produce total gastric atrophy, the structural impairment of parietal cells over-rides the functional one so that no return of acid in achlorhydric subjects is to be expected.

The clinical division of such patients may be important for two reasons. Firstly, some of the achlorhydric patients with antibodies to gastric parietal cells who present with iron deficiency anaemia, may proceed to pernicious anaemia. Evidence for this is lacking at the present time and it will be forthcoming only by a careful follow-up study of such patients. Secondly, it is probable that iron absorption is impaired in the achlorhydric patient, and so this group of achlorhydric iron deficient patients may be more difficult to treat than those patients who are not achlorhydric. The report by Gibson, Kelly and Wang (1963) suggested that some degree of iron deficiency is significantly more common in patients with pernicious anaemia (i.e. achlorhydric patients) who have been treated with vitamin B_{12} alone, than in normal controls. It seems probable that the fact that most of these patients are post-menopausal and have only limited iron requirements prevents the occurrence of frank iron deficiency anaemia more commonly.

SUMMARY OF SECTION I

A method of inducing severe iron deficiency anaemia in the rat is described. Pregnant female rats were placed on an iron deficient diet and after gestation, the infant rats were maintained on the same iron deficient diet and killed 28 and 42 days after birth. The rats showed severely retarded growth and marked iron deficiency anaemia with haemoglobin levels as low as 1.0 g%. In these rats, histological and histochemical studies of the liver revealed severe central zone damage with a decrease in succinic dehydrogenase and an increase in fat in these areas. There were only minimal or inconsistent histological and histochemical abnormalities in the stomach, small intestine and colon. Gastric acid studies were carried out on pylorus ligated rats with iron deficiency anaemia prepared by the above method, the haemoglobin levels being in the range 2.7 to 9.8 g%. Impairment of gastric acid secretion was not demonstrated.

Gastric function was studied in 17 patients with moderate to severe iron deficiency anaemia. In those patients with idiopathic iron deficiency anaemia, there was a high incidence of achlorhydria and gastritis with circulating parietal cell antibodies in the peripheral blood. None of these patients had antibodies to intrinsic factor. In those patients in whom anaemia was due to chronic blood loss, the gastric secretion of hydrochloric acid was reduced but was not usually absent and gastric parietal cell antibodies could not be detected. The acid secretion in some patients had increased when they were retested several months after the correction of anaemia. An improvement

in secretion after treatment was also seen in patients with anaemia due to folic acid deficiency. In some patients with iron deficiency the gastric acid response to a histamine dose of 0.1 mg/kg. was greater than the response to 0.04 mg/kg. suggesting that the parietal cell in iron deficiency may be less responsive to stimulation. As a result of these studies, it is suggested that patients with iron deficiency anaemia and gastritis may be divided into two groups depending on whether the gastric mucosal lesion precedes or follows the anaemia.

SECTION 2 - THE RELATIONSHIPS BETWEEN ANAEMIA AND CARCINOMA OF THE UPPER GASTROINTESTINAL TRACT

INTRODUCTION

Gastric Function, Gastritis and Pernicious Anaemia in Gastric Carcinoma

The mortality from carcinoma of the stomach varies widely from country to country but the number of cases is decreasing in some countries, for example, in the U.S.A. (Haenszel 1958) and in England and Wales (Case 1956, Brookes, Waterhouse and Powell 1965) while it is rising in others, for example in Japan (Segi and Kurihara 1960). Such epidemiological studies do suggest that diet and other exogenous factors might play some part in these variations. Nevertheless, it is known that hereditary factors are also important in the genesis of the disorder as is reflected in the high incidence of carcinoma of the stomach in relation to pernicious anaemia (Kaplan and Rigler 1945 and Mosbech and Videbaek 1950) and in relation to other cases of carcinoma of the stomach (Maimon and Zinninger 1953, Videbaek and Mosbech 1954, Woolf 1956, Savage 1956, Graham and Lilienfeld 1958 and Sommers 1958). In addition, cases of carcinoma of the stomach show a bias in favour of Blood Group A (Aird, Bentall and Roberts 1953) which is likely to be due to the blood group A bias in pernicious anaemia (Hoskins, Loux, Britten and Zamcheck 1956).

In 1876, Quincke described a patient with both pernicious anaemia and carcinoma of the stomach. Since then, there have been various

estimates of the incidence of carcinoma in pernicious anaemia. In a series of autopsy cases of pernicious anaemia, the incidence varied from 0.7% to 12.9% with an overall incidence of 5.2% (See Table 17). Clinical studies based upon the follow-up of patients with pernicious anaemia gave a cancer incidence which varies from 0.6% to 7.5% (see Table 18) with an overall incidence of 2.3%.

In a more recent study, Blackburn, Callander, Dacie, Doll, Girdwood, Mollin, Saracci, Stafford, Thompson, Varadi and Wetherley-Mein 1968) of 1625 patients with pernicious anaemia there were 29 deaths from gastric carcinoma as against 7.3 expected deaths over a 4 year period.

There is a male preponderance of carcinoma of the stomach in all countries, but pernicious anaemia is more common in women. However, males who do have pernicious anaemia are more likely to get gastric carcinoma. For example, in a series of 1222 cases of pernicious anaemia (Zamcheck, Grable, Ley and Norman 1955) there were 16 males and 12 females with gastric cancer although the sex ratio for pernicious anaemia was 3 females to every 2 males. The Mayo Clinic records (Schell, Docherty and Comfort 1954) of patients who had both disorders show a male to female ratio of 2.5:1.

It has been stated that in pernicious anaemia the tumour site distribution may differ from that in cases without pernicious anaemia. Schell, Grable, Lay and Norman (1954) state that there is a greater tendency for the tumour to be in the body or fundus of the stomach and only about a third occur in the pylorus and antral area. Gastric polyps are also likely to occur in pernicious anaemia and in some cases the carcinoma may

Table 17. The incidence of carcinoma of the stomach in pernicious anaemia as determined from autopsy studies (from Chanarin 1969).

PERNICIOUS ANAEMIA

Author	No. of cases	Carcinoma	% with carcinoma
Brown (1934)	151	1	0.7
Strandell & Jansson (1937)	686	26	3.8
Kaplan & Rigler (1945)	293	36	12.3
Böttner (1946)	49	3	6.1
Kade (1949)	259	12	4.6
Swynnerton & Truelove (1952)	375	4	1.1
Doehring (1954)	81	6	7.4
Zamcheck et al (1955)	167	14	8.4
Cappell (1957)	54	7	12.9
Total	2115	109	5.2

TABLE 18

The incidence of carcinoma of the stomach in pernicious anaemia as determined from clinical studies (from Chanarin 1969)

	PERNICIOUS ANAEMIA		
Author	No. of cases	Carcinoma	% with carcinoma
Strandell (1931)	117	4	3.4
Connor & Birkeland (1933)	658	4	0.6
Murphy & Howard (1936)	440	4	0.9
Washburn & Rozendaal (1938)	906	16	1.8
Jenner (1939)	181	7	3.9
Coester (1941)	149	7	4.7
Doehring & Eusterman (1942)	1014	17	1.7
Jankelson et al (1943)	100	4	2.5
Frank (1944)	188	5	2.7
Waldenstrom (1945)	233	14	6.0
Christoffersen & Clausager-Madsen (1946)	103	2	1.9
Wallace (1948)	203	2	1.0
Wilkinson (1949)	1532	28	1.8
Jorgensen (1951)	206	12	5.8
Mosbech & Videbaek (1950)	301	15	5.0
Norcross et al (1952)	341	4	1.7
Doehring (1954)	432	8	1.8
Hitchcock et al (1955)	77	4	5.2
Zamcheck et al (1956)	1222	28	2.3
Berkson et al (1956)	221	8	3.6
Siurala et al (1959)	69	5	7.5
Rousso & Cruchaud (1966)	54	2	3.7
Total	8747	200	2.3

Table 18.

arise from these (Eklof, Engstedt and Reizenstein 1962).

Achlorhydria and hypochlorhydria are common in carcinoma of the stomach and they may exist for many years before the development of a tumour (Hitchcock, Sullivan and Wangenstein 1955, Comfort, Kelsey and Berkson 1956, Berkson, Comfort and Butt 1956 and Schade 1958). Hitchcock, Maclean and Sullivan (1957) studied 3439 achlorhydric and hypochlorhydric patients for up to 10 years and found that 1.1% developed carcinoma of the stomach. This incidence was much higher than the frequency of carcinoma of the stomach in the general population of the same age group. In general, the incidence of achlorhydria is approximately the same whatever the site of the tumour (McNeer and Pack 1967).

Since gastritis is usually an accompaniment of achlorhydria it might be expected that there would be an increased incidence of carcinoma in patients with proven gastritis. This has been confirmed. In 53 patients with atrophic gastritis (Siurala and Seppala 1960), 5 developed carcinoma in a six year follow-up. When followed up for ten to fifteen years, 9 out of 116 patients had developed gastric cancer (Siurala, Eramaa and Tapiovaara 1959). In 93 patients with superficial gastritis and 168 with a normal gastric mucosa no cancer developed.

Carcinoma of the stomach is more common amongst the relatives of pernicious anaemia patients (Mosbech 1953 and Siurala, Eramaa and Tapiovaara 1959) - in both series the incidence was 3 times greater than would have been expected. Similarly, there is a higher incidence of pernicious anaemia among relatives of patients with carcinoma of

the stomach (Macklin 1955) Thus carcinoma of the stomach and pernicious anaemia are both associated with achlorhydria and atrophic gastritis and they both show a familial incidence.

The present investigations were undertaken to further assess the relationship between gastric carcinoma and the achlorhydria which occurs in pernicious anaemia (Shearman, Finlayson, Wilson and Samson 1966). The secretion by the stomach of acid, pepsin and intrinsic factor in existing cases of carcinoma of the stomach has been studied on the basis that any abnormalities that were detected might exist in the premalignant state (Shearman, Finlayson and Wilson 1967). These features could then be looked for in prospective studies of patients with gastritis and achlorhydria.

CARCINOMA OF THE OESOPHAGUS AND ANAEMIA

Exogenous factors in the aetiology of carcinoma of the oesophagus

The wide variation in the incidence of the disease in different countries has been interpreted as due to differing exposure to environmental factors. The main associations are with smoking, alcohol and with tobacco and betel nut chewing. For example, the South African distribution of the disease follows that of lung cancer which has a known association with smoking (Oettle 1963). Groups of people who do not smoke were found to have a low incidence of the cancer (Wynder, Lemon and Bross 1959) and the high incidence in smokers is present whether or not they inhale, suggesting that the carcinogen is swallowed (Schwartz, Flamant, Lellouch and Denoix 1961). There is also a fairly definite association with alcohol consumption, particularly if the alcohol is contaminated as with many home-brews, for example by brewing in lead plated kerosene tins (Oettle 1961). Nevertheless, the incidence is also higher in peoples of Northern countries who drink alcohol (Schwartz, Lellouch, Flamant and Denoix 1962). Regional differences in France are clearly correlated with mortality from cirrhosis, thus supporting an association with alcohol (Lasserre 1963).

Anaemia and post-cricoid carcinoma

The Paterson-Kelly Syndrome - This syndrome of anaemia, glossitis and dysphagia was described by Paterson (1919) and Kelly (1919) and it occurred predominently in women. This large female preponderance has been emphasised with 90 of 94 cases reported by McNab Jones (1961) and 53 of 55 cases reported by Jacobs and Kilpatrick (1964) being females. The syndrome is

also associated with achlorhydria. In 10 patients tested by Moersch and Connor (1926), 8 were found to be achlorhydric and in addition the atrophic appearance of the mucosa of the mouth, pharynx and oesophagus was noted. Witts (1931) also noted the association with achlorhydria and it is now generally accepted that gastric achlorhydria is likely to be part of the syndrome (Whitby and Britton 1957).

There is discussion as to whether the dysphagia arises as a result of oesophageal epithelial changes which are secondary to the iron deficiency or whether the dysphagia can occur without iron deficiency. In this respect Wynder and Fryer (1958) found normal serum iron levels in 68% of a group of women with the Paterson-Kelly syndrome. Similar results were reported by Jacobs (1962). Elwood, Jacobs, Pitman and Entwistle (1964) compared the incidence of iron deficiency in patients with dysphagia, some of whom had a pharyngeal web, with a control group, and no difference was found. Others (Witts 1931, Waldenstrom and Hallen 1938) believe that the iron deficiency anaemia precedes the onset of dysphagia. Patients have been described (Chisholm and Wright 1967) who were not iron deficient at the time of diagnosis but who had had severe iron deficiency in the past.

Post-cricoid carcinoma and the Paterson-Kelly Syndrome

Both Paterson (1919) and Kelly (1919) noted the association of this syndrome with carcinoma of the upper oesophagus. Simpson (1939) followed up 18 patients with the Paterson-Kelly Syndrome and found that 10 developed oesophageal carcinoma (post-cricoid 4 cases, lower oesophagus 1 case

and gastro-oesophageal junction 5 cases). Five out of 28 females with anaemia and dysphagia developed post-cricoid carcinoma in 15 years. (Owen 1950). Ahlbom (1936) stated that 70% of female patients with cancer of mouth, pharynx or oesophagus had either Paterson-Kelly Syndrome or iron deficiency anaemia and Lederman (1958) found that epithelial signs of iron deficiency were common in patients with post-cricoid carcinoma. As with the Paterson-Kelly Syndrome there is a female preponderance in post-cricoid carcinoma (Jacobs 1961).

The Pathology of this Association

The mechanism whereby iron deficiency might lead to tissue damage is uncertain and the lack of enzymes which contain iron is one possibility (Beutler 1957) Iron deficiency might also initiate an autoimmune process (Moulton 1964, Wright 1965). Once atrophy of the mucosa has occurred it has been implied that it represents a premalignant condition (Welin 1953, Boyd 1961). Many workers (Paterson, 1919, Suzman 1933, Savilahti 1946 and Palmer 1952) have noted thinning of the oral and oesophageal mucosa in iron deficiency but the only definitive study on oesophageal epithelium was carried out by Jacobs (1961) who compared patients with post-cricoid carcinoma to those with carcinoma higher in the pharynx. He found that post-cricoid cancer was not usually associated with the mucosal changes found in anaemic patients even when there was a history of anaemia. The post-cricoid web itself does not appear to be premalignant since when the carcinoma does develop it is not often in the web (Welin 1953). Thus the significance of post-cricoid or upper oesophageal web in the aetiology of

post-cricoid carcinoma is uncertain and the patients with webs are not necessarily the anaemic ones since only 12 of 46 patients with webs were found to have anaemia (Shammaa and Benedict 1958).

Dysphagia, pernicious anaemia and post-cricoid carcinoma

Dysphagia in association with pernicious anaemia has been noted in the past (Jones and Owen 1928, Croskery 1928, McGibbon 1935). Simpson (1939) reported a case of post-cricoid carcinoma in a patient with pernicious anaemia and in 1820 cases of pernicious anaemia Wilkinson (1950) found 11 cases of buccopharyngeal carcinoma. Jacobs (1961) notes some similarity in England and Wales between the distribution of post-cricoid carcinoma and the distribution of pernicious anaemia.

The association of oesophageal cancer and other disorders

The above associations apply particularly to post-cricoid cancer but other associations are seen with oesophageal carcinoma at any site.

Achalasia

The incidence of carcinoma in achalasia is greater than in persons with a normal oesophagus and varies from 3-8% (Bockus 1963). The tumour can occur at any site within the oesophagus.

Hiatus Hernia

Smithers (1955) noted an increase in the incidence of oesophagogastric cancer in association with hiatus hernia. In general, this association is impossible to analyse since the definition of hiatus hernia used in the literature is not strict and most papers do not distinguish between adeno and squamous carcinoma, so that many of the reported tumours may

may be gastric cancers.

Nutritional deficiency

The high incidence of oesophageal carcinoma in parts of Africa (Hutt and Burkitt 1965) has been cited as evidence for nutritional deficiency as a cause. In addition, squamous carcinoma of the thoracic oesophagus has been reported in 4 cases of proven malabsorptive disease (Wright and Richardson 1967). One of these patients had life-long coeliac disease and developed the tumour at the age of 23. Another patient was a female aged 38. In all four cases, the tumour was in the lower two-thirds of the oesophagus and three of the four had suffered from megaloblastic anaemia at some time in the past.

Gastric or duodenal ulcer

Very little attention has been paid to this problem although Wright and Richardson (1967) in a 5 year period survey of 41 men and 26 women with cancer of the thoracic oesophagus treated at the London Hospital, note that 12% of the patients had long standing dyspepsia. Two of these patients had undergone gastric surgery for duodenal ulcer 34 and 12 years previously. One had surgery for gastric ulcer 4 years previously and one had a total gastrectomy for pyloric carcinoma 13 years prior to the oesophageal tumour.

PATIENTS AND METHODS

Patients

Patients who were admitted to the Royal Infirmary and in whom carcinoma of the stomach was diagnosed were investigated. Seventy consecutive patients were studied, in 60, the diagnosis was proved at operation, with histological evidence in 42 of them. In 10 patients, the cancer was judged to be too advanced for surgery and the radiological diagnosis was accepted : in one of these latter cases, adenocarcinoma cells were found on gastric lavage.

Of the 70 cases of gastric carcinoma, all were studied for antibodies to parietal cells and to intrinsic factor, 55 had measurements of gastric acid production, and 41 had intrinsic factor measurements. In 32 of the 55 cases, the tumour was in the body of the stomach and in 18, it was antral. Five tumours were so extensive that classification was impossible.

Ninety-two consecutive patients with squamous carcinoma of the oesophagus seen at the Radiotherapy Unit in Edinburgh were investigated. These form the majority of patients with this disease in the South-Eastern Region of Scotland over a period of $1\frac{1}{2}$ years from July, 1967 to December 1968 because only 10 patients were treated initially by surgical resection during this period. (Pearson - personal communication). The Edinburgh Radiotherapy and Thoracic Surgery Departments serve a relatively closed population of approximately one and one third million and so the series of 92 patients is likely to include a majority of patients with cancer of the oesophagus occuring in South-East Scotland (Pearson 1966). The site of

the tumour was classified as the distance from the upper incisor teeth to the upper margin of the tumour (Pearson 1966). In addition, for statistical purposes, the tumour was defined as upper (above 18cm.) middle (18-29cm.) or lower (30cm. or greater). All patients were interviewed at the time of hospitalisation for radiotherapy. Inevitably some patients were overlooked or died before they could be interviewed and the information on these patients was completed from General Practice and hospital case records. On interviewing the patient, the particular points which were sought are listed in Table 19.

Methods

Blood was taken from all patients before operation or radiotherapy for routine haematological assessment, blood grouping and measurements of serum iron, iron binding capacity, serum vitamin B_{12} and parietal cell and intrinsic factor antibodies. The methods used were those described earlier. Gastric function tests were carried out as before, and the pentagastrin dose of 6 μg/kg body weight was regarded as an equivalent stimulant to histamine for both acid (Multicentre Pilot Study 1967) and intrinsic factor (Shearman, Finlayson, Murray-Lyon, Samson and Girdwood 1967). For intrinsic factor estimation samples were collected on to ice at ten minute intervals and immediately neutralised, that is, after the samples were taken for pepsin and acid estimation, the remaining aliquot was rapidly titrated to pH 10 and allowed to stand for 20 minutes to destroy all peptic activity. The pH of the samples were then returned to 7 and the specimen frozen and stored for the

Table 19. Patient interviews.

The following associations were sought:-

1. Previous dysphagia and duration i.e. ? Achalasia, ? Paterson-Kelly Syndrome.

2. Previous anaemia or treatment with haematinics.

3. History of, or investigation for hiatus hernia.

4. Previous history, investigation or operation for peptic ulcer.

5. History of thyroid disease, diabetes mellitus, Addisons Disease.

6. Previous irradiation.

7. Other malignancies.

estimation of intrinsic factor. Intrinsic factor was measured by the charcoal method (Ardeman and Chanarin 1963). Repeat estimations were made on all samples and any discrepancies were followed by a repeat of the estimation until a constant reading was obtained. Pepsin estimations were carried out as before. For comparative purposes, patients with gastric ulcer, duodenal ulcer and patients without gastric disorder underwent augmented histamine secretion tests and estimates of acid, pepsin and intrinsic factor were made.

In-vitro intrinsic factor studies were carried out for the detection of inhibitors in the gastric juice of carcinoma patients. In these, gastric juice from cases of duodenal ulcer with a high titre of intrinsic factor per mEq. of acid was added to juice from gastric carcinoma patients. The samples were then assayed for intrinsic factor as before.

Since it is not possible to intubate the stomach of the majority of patients with oesophageal carcinoma, gastric acid secretion was assessed indirectly by an Azuresin test (Segal 1960). This involves the oral administration of a resin indicator compound, the azure being liberated in the presence of free gastric hydrochloric acid. The azure A released from the resin is then absorbed and excreted in the urine where it can be detected. In the present study, the azure A present in the urine was measured colorimetrically by reference to a standard curve. If the colour intensity of the test urine was equal to or exceeded that of a 0.6 mg. standard, then free hydrochloric acid had been secreted from the stomach and when the colour was less than that of a 0.3 mg. standard, this was regarded as evidence of achlorhydria.

RESULTS

GASTRIC CARCINOMA

Gastric function and tumour site

Thirty-two patients with carcinoma of the body of the stomach (20 males and 12 females) and 18 with antral carcinoma (9 males and 9 females) had measurements of acid secretion. Figure 12 shows the individual values and the mean values for these patients. Sixteen with body carcinoma and 5 with antral carcinoma were achlorhydric. One patient with body carcinoma and 5 with antral carcinoma produced more than 10 mEq of acid in the post-histamine hour. There was no statistically significant difference either in the proportion of achlorhydric patients or in the mean values of the acid produced by the patients in the 4 groups. There was no relation between acid production and the local extent of the tumour in the first 22 patients studied (see Tables 20 - 22). For example, very small tumours were found in some achlorhydric cases and fairly large tumours were present in some patients who could produce acid.

Twenty-one patients with body carcinoma (13 males and 8 females) and 16 with antral carcinoma (7 males and 9 females) had measurements of intrinsic factor secretion in the hour after stimulation. Fig. 13 shows the individual values and mean values for these patients. There were no significant differences between body and antral groups and there was no relation between intrinsic factor production and the local extent of the tumour (Tables 20 - 22).

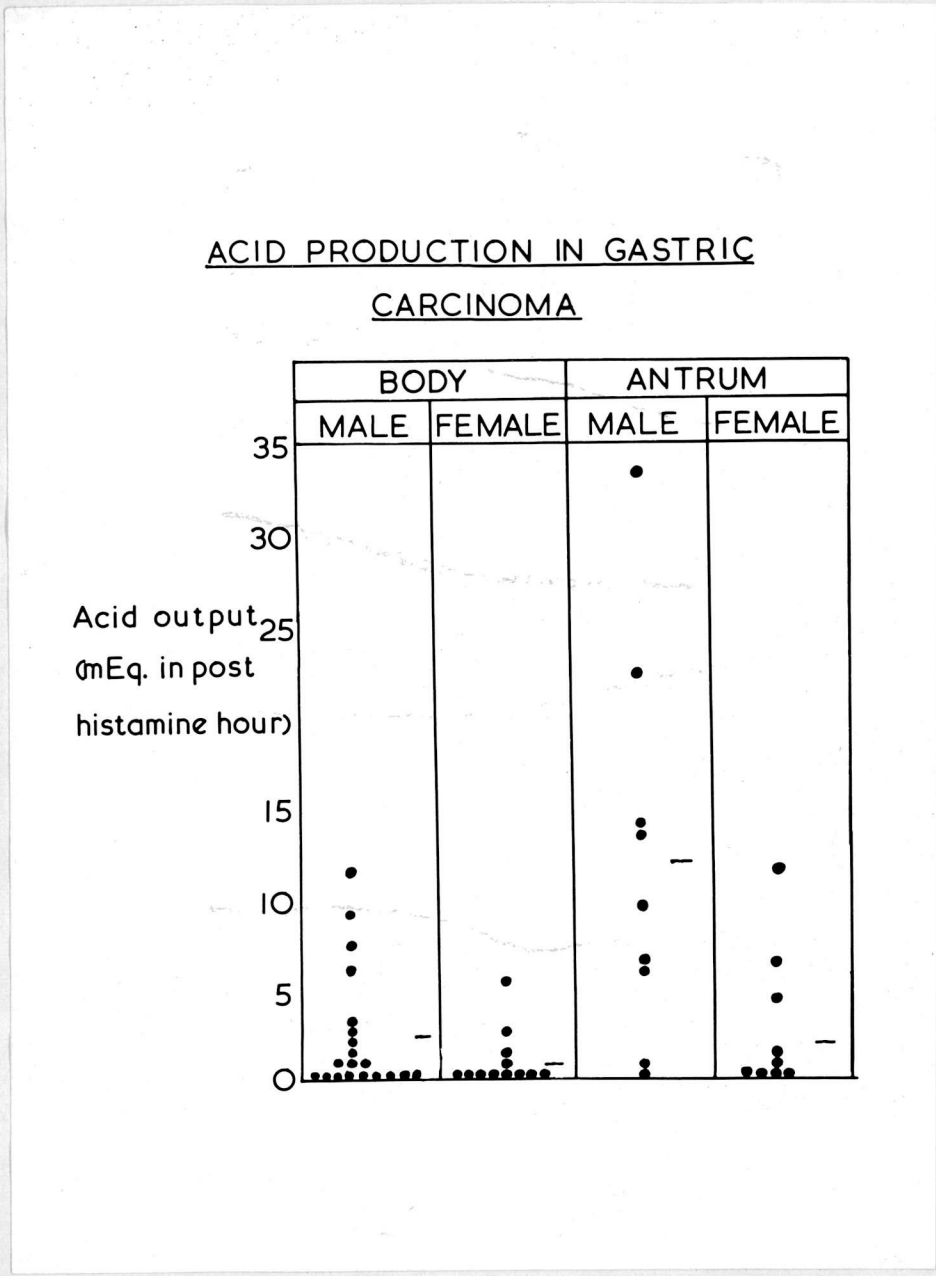

Figure 12. Acid production in patients with gastric carcinoma.

ACHLORHYDRIC GASTRIC CARCINOMA PATIENTS

AGE	GASTRIC FUNCTION		SEROLOGY		TUMOUR		
	ACID OUTPUT	I.F. OUTPUT	PARIETAL CELL ANTIBODY	I.F. ANTIBODY	SITE	EXTENT	HISTOLOGY
56	NIL	1824	—	—	PYLORUS	ULCER 2·5x1·5 cms.	ANAPLASTIC
68	NIL	1681	—	0·68	BODY	MOD. EXTENSIVE	ANAPLASTIC
77	NIL	852	—	1·05	MID BODY		
72	NIL	793	—	—	WHOLE	STOMACH	—
85	NIL	248	++	—	PYLORUS		
73	NIL	44	+	0·05	BODY	WHOLE LESSER CURVE	ANAPLASTIC*

* NORMAL MUCOSA ATROPHIC

Table 20. Gastric function studies, serology and tumour features in achlorhydric gastric carcinoma patients.

ACID PRODUCING GASTRIC CARCINOMA PATIENTS

AGE	GASTRIC FUNCTION		TUMOUR FEATURES		
	ACID OUTPUT	INTRINSIC FACTOR OUTPUT	SITE	EXTENT	HISTOLOGY
50	33·48	8481	PYLORUS	MASS 5x4 cms.	ANAPLASTIC
64	14·10	9022	PYLORUS	MASS 2x3 cms.	ADENO-CARCINOMA
64	11·29	3350	BODY	LESSER CURVE	–
60	11·25	3969	ANTRUM	ANTRUM ONLY	–
67	9·73	7809	PYLORUS	ULCER 2x2 cms.	ANAPLASTIC
70	6·44	2577	PYLORUS	SMALL ULCER	–
59	6·04	2928	PYLORUS	WHOLE PYLORUS	ANAPLASTIC
67	5·8	4221	BODY	VERY EXTENSIVE	ADENO-CARCINOMA
60	2·44	5505	BODY	SMALL ON BARIUM MEAL	–

Table 21. Gastric function studies and tumour features in patients with gastric cancer who produced acid.

ACID PRODUCING GASTRIC CARCINOMA PATIENTS.

AGE	GASTRIC FUNCTION		TUMOUR FEATURES		
	ACID OUTPUT	INTRINSIC FACTOR OUTPUT	SITE	EXTENT	HISTOLOGY
39	1·92	3288	PYLORUS BODY	VERY EXTENSIVE	ANAPLASTIC
70	1·39	1004	BODY	POSTERIOR WALL	—
60	1·26	4935	BODY	SMALL (Barium meal)	—
61	0·58	968	ANTERIOR WALL	MASS 7x7 cms.	ADENO-CARCINOMA
73	0·43	3643	BODY	POSTERIOR WALL	ANAPLASTIC
28	0·38	3461	BODY	LESSER CURVE and POST. WALL	—
54	0·02	441	DISTAL BODY	MASS HALF BODY	ANAPLASTIC

Table 22. Gastric function studies and tumour features in patients with gastric cancer who produced acid.

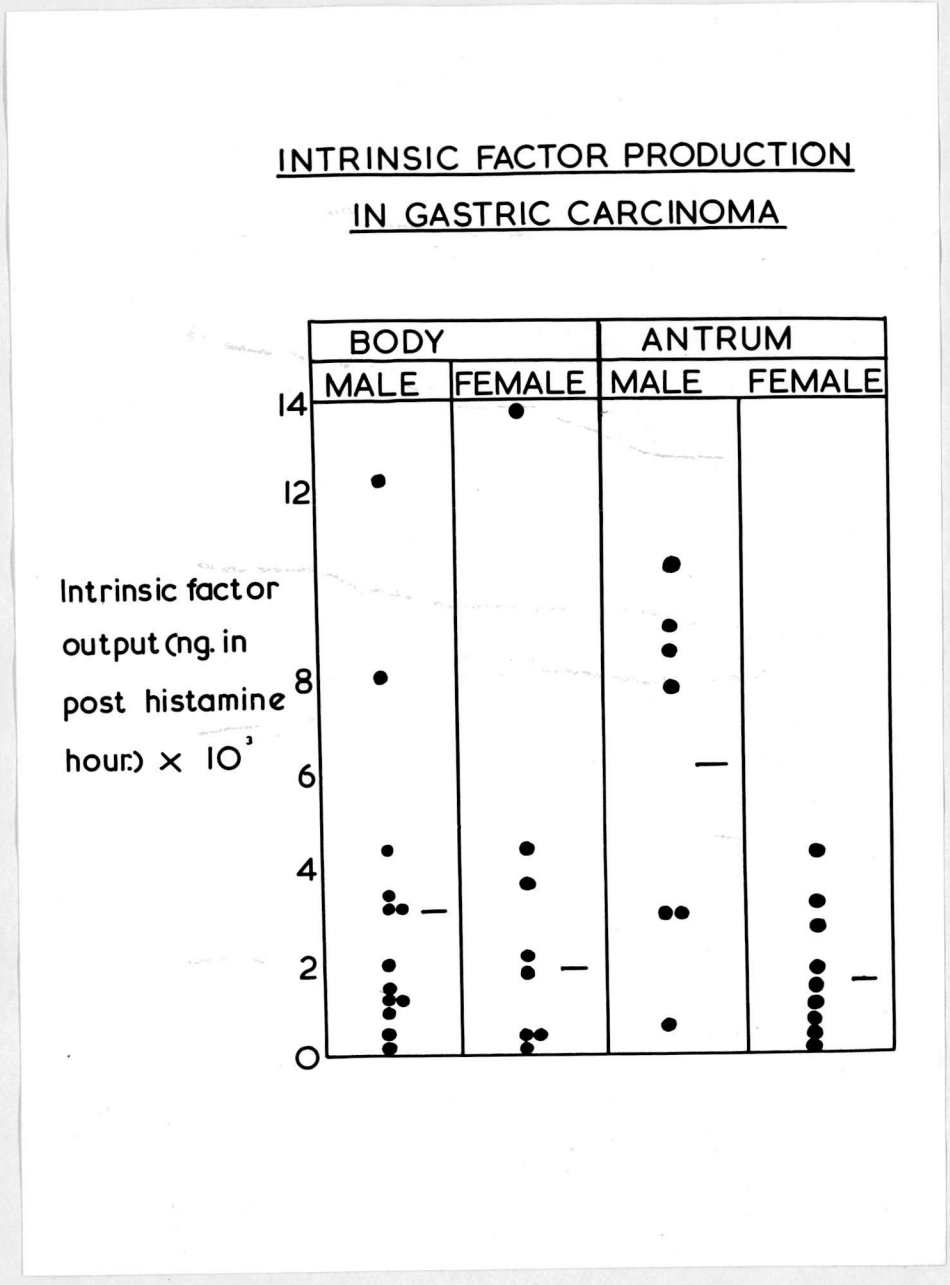

Figure 13. Intrinsic factor production in patients with gastric carcinoma.

In the earlier part of the study, the acid and intrinsic factor estimations on 22 patients with gastric carcinoma were compared with these estimations in patients with gastric ulcer, duodenal ulcer and patients without a gastric disorder (Figs 14 and 15). The mean acid output in gastric carcinoma patients was 4.9 mEq. (range 0-33 mEq). The mean acid output for patients with gastric ulcer was 14.4 mEq. (range 3.4-34.4 mEq). The difference between these groups is significant ($P < 0.05$). The mean acid in subjects without gastric disorder and in persons with duodenal ulcer was 18.7 mEq (range 9.0-39.0 mEq) and 33.1 mEq (range 10.8-66.0 mEq) respectively.

Fig. 15 shows the intrinsic factor outputs in ng. units during the post-histamine hour in these 22 patients. The mean intrinsic factor output for gastric carcinoma patients was 3230 ng. units (range 4444-9022 ng. units). The mean output for gastric ulcer patients was 12,840 ng. units (range 6026-23,097 ng. units). The difference between these groups was statistically significant ($P < 0.001$). The mean intrinsic factor output in the subjects without gastric disorder and in persons with duodenal ulcer was 14,800 ng. units (range 6000-31,800 ng. units) and 22,954 (range 10,900-32,900 ng. units).

Fig. 16 shows the relation between intrinsic factor output and acid output in the post-histamine hour in the 22 patients with gastric carcinoma described above. There was a direct relationship between acid and intrinsic factor output in all patients but certain individual carcinoma patients who could produce acid appeared to have a relative deficiency of intrinsic factor secretion.

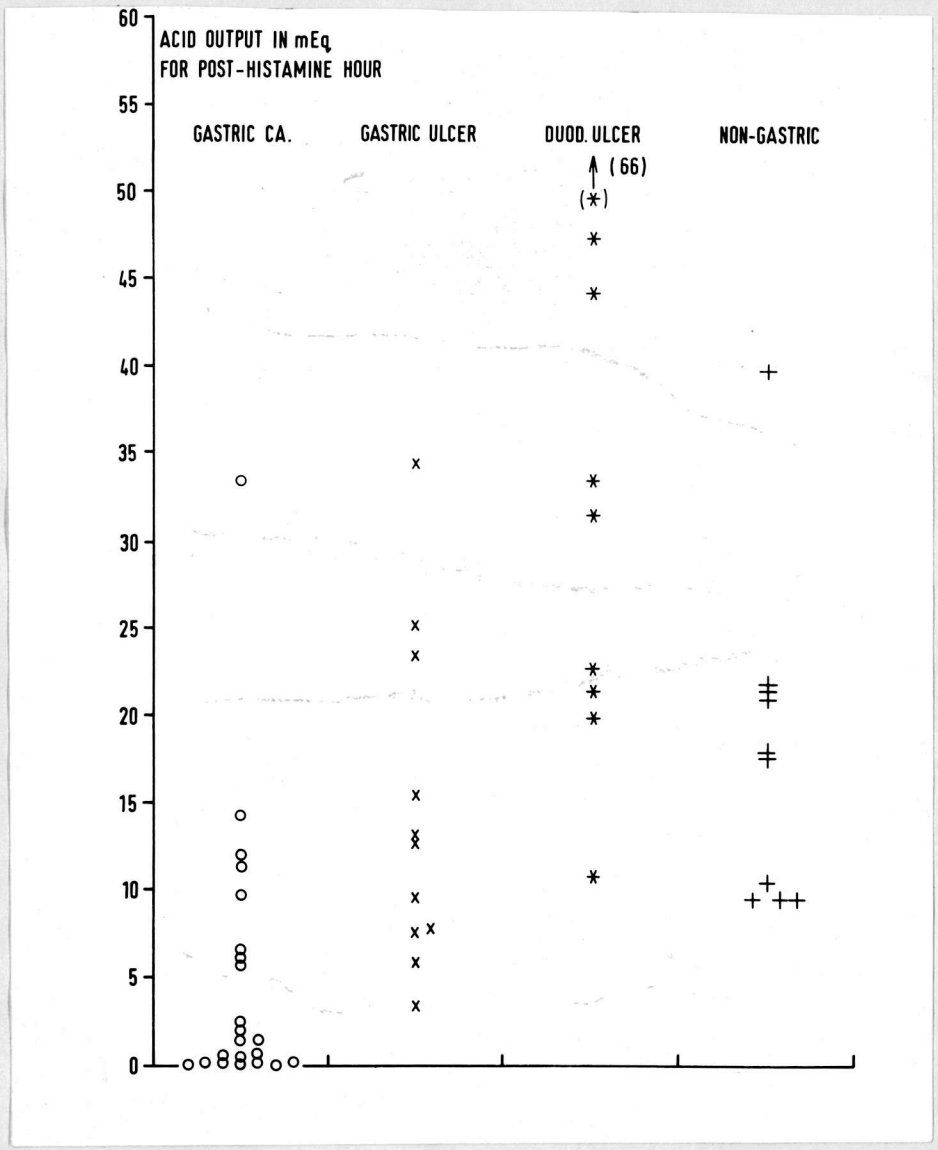

Figure 14. Acid output in patients with gastric cancer, gastric ulcer, duodenal ulcer and patients with no gastric disorder.

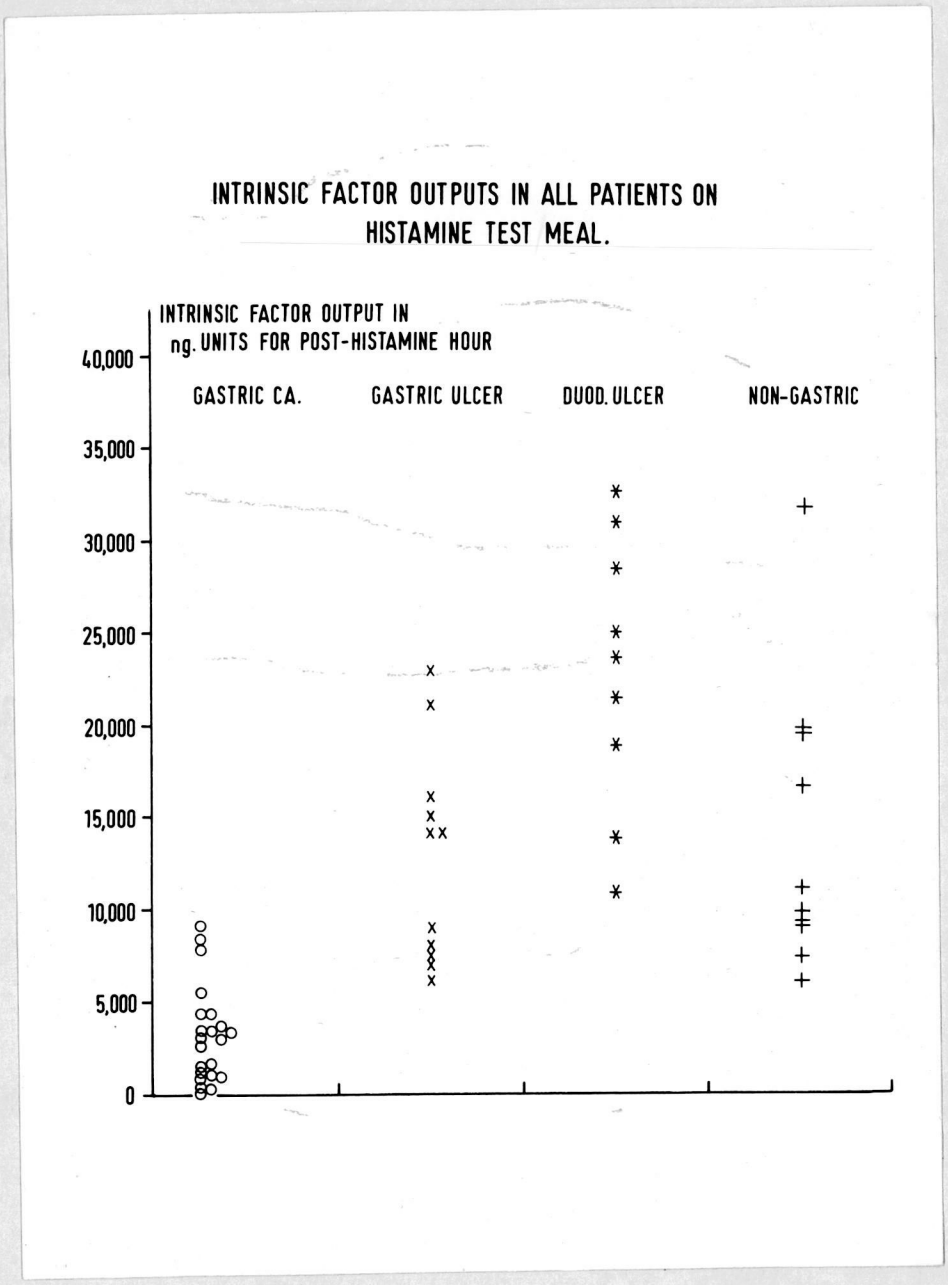

Figure 15. Intrinsic factor output in patients with gastric cancer, gastric ulcer, duodenal ulcer and patients with no gastric disorder. (The same patients as in Figure 14.).

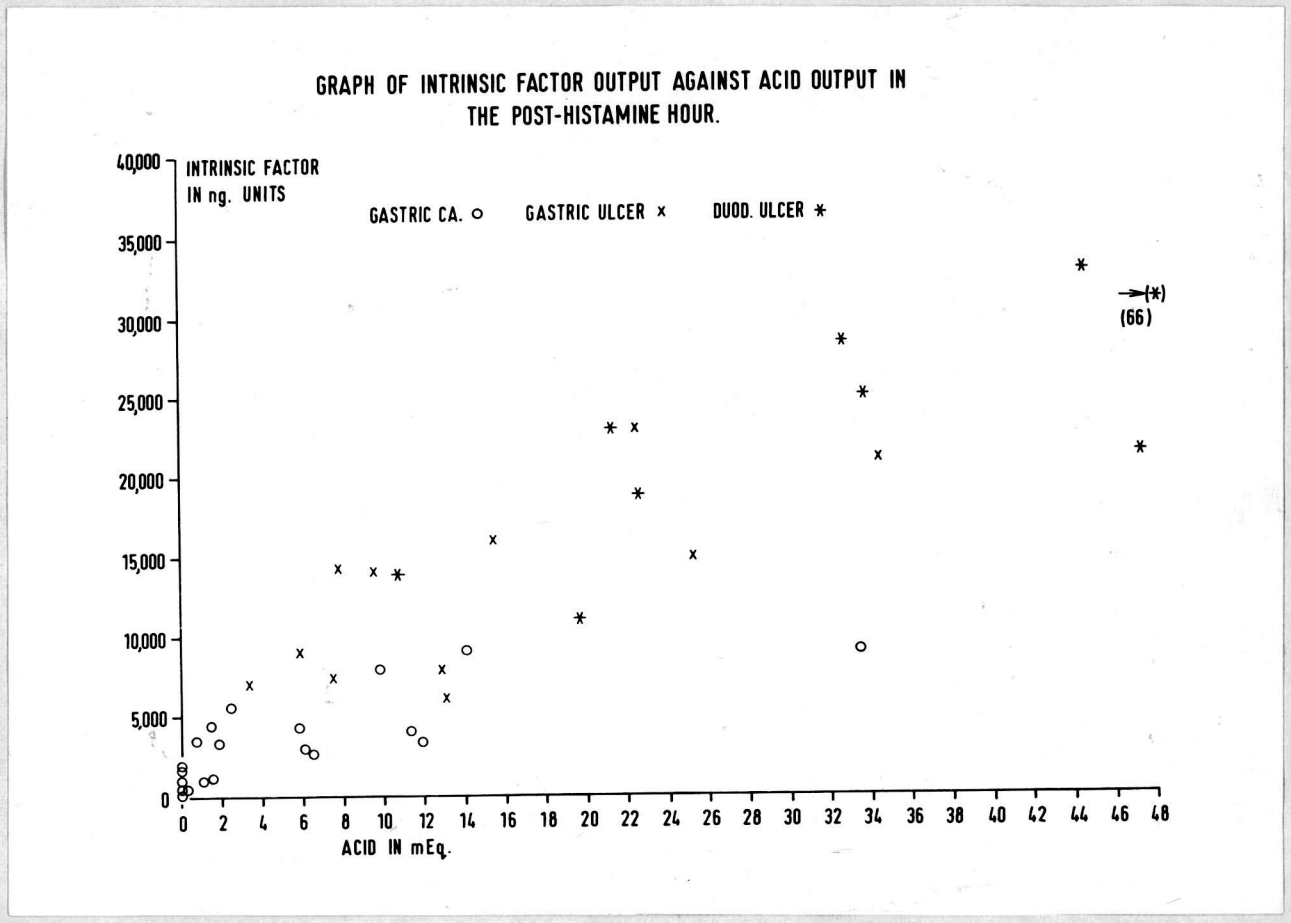

Figure 16. Comparison of intrinsic factor and acid outputs in patients tabulated in Figures 14 and 15.

In-vitro studies on the gastric juice of gastric carcinoma patients are summarised in Table 23. It will be seen that patients 1-3 with duodenal ulceration have a higher concentration of intrinsic factor in ng. units/ml. of gastric juice than do gastric carcinoma patients 4-6. The relationship between intrinsic factor and acid shown in Fig. 16 is reflected in the figures of 280, 409 and 330 ng. Units/mEq. acid of the carcinoma patients. It will be seen that combined assay of juice from gastric carcinoma and duodenal ulcer produces the predicted result indicating lack of inhibition.

Table 24 shows the secretory findings in the 5 patients with extensive involvement of the gastric mucosa.

Gastric Antibodies

Of the 70 patients, 4 had both parietal cell antibody and intrinsic factor antibody, 6 had parietal cell antibody and 2, in one of whom parietal cell antibodies were not looked for, had intrinsic factor antibody. Table 25 shows those in the series who were already known to have pernicious anaemia (patients 1 - 4). They were receiving regular treatment, and it will be seen that one had neither parietal cell antibodies nor intrinsic factor antibodies : patient 5 presented with a gastric carcinoma and was found to have pernicious anaemia in addition. Table 26 shows the remaining patients who had one or other gastric antibody. On the basis of the evidence shown in Table 26 patients 6 - 8 , none of whom were anaemic, are considered to have latent pernicious anaemia, defined as malabsorption of vitamin B_{12} reversible with

CASE No.	DIAGNOSIS.	INTRINSIC FACTOR CONTENT.	
		mg. UNITS/ML.	mg. UNITS/mEq.
1.	DUODENAL ULCER	119	1656
2.	DUODENAL ULCER	58	663
3.	DUODENAL ULCER	94	1798
4.	GASTRIC CARCINOMA	20	280
5.	GASTRIC CARCINOMA	18	409
6.	GASTRIC CARCINOMA	20	330

CASE No.	PROPORTION OF EACH	INTRINSIC FACTOR mg. UNITS/ML.	
		POSSIBLE	ACTUAL
1+5	1/2	69	75
3+6	1/2	57	52
4+6+2+3	1/4	48	42

N.B. CASES 4, 5 AND 6 SHOWED MINIMUM INTRINSIC FACTOR OUTPUT / mEq. OF ACID.

Table 23. In vitro studies for the detection of inhibitors in the gastric juice of carcinoma patients who showed a low intrinsic factor output per mEq. of acid in the gastric secretion.

Table 24. Acid and intrinsic factor production in extensive gastric carcinoma

ACID (mEq in post-histamine hour)	INTRINSIC FACTOR (ng in post-histamine hour)
0.0	-
0.0	653
0.0	793
1.9	3288
6.7*	7938

* Linitis plastica.

Table 25. Patients with frank pernicious anaemia and gastric carcinoma.

No.	Age	Sex	Antibodies		Serum Vitamin B_{12} (pg/ml)	Carcinoma Site
			P.C.A.	I.F.A.		
1	48	F	-	-	Treated	Body
2	68	M	N.D.	++	Treated	Body
3	72	F	+	++	Treated	Body
4	74	F	+	+	Treated	Body
5	85	F	++	-	69	Antrum

P.C.A. = Parietal cell antibody.

I.F.A. = Intrinsic factor antibody.

N.D. = Not done.

Table 26. Gastric carcinoma patients with autoimmune antibodies.

No.	Age	Sex	Antibodies P.C.A.	Antibodies I.F.A.	Serum (pg/ml)	Vitamin B_{12} Absorption % Alone	Vitamin B_{12} Absorption % + I.F.	Stomach Acid	Stomach I.F.	Carcinoma Site
6	56	M	-	+	120	4.3	15.0	0	N.D.	Antrum
7	66	M	++	++	42	0	10.9	0	N.D.	Body
8	73	M	+	-	48	N.D.	N.D.	0	44	Body
9	85	F	++++	+	1000	N.D.	N.D.	1.0	N.D.	Body
10	70	F	+++	-	928	N.D.	N.D.	N.D.	N.D.	Body
11	72	F	++	-	164	N.D.	N.D.	0	433	Body
12	78	F	++	-	216	N.D.	N.D.	0.5	410	Antrum
13	70	F	+	-	365	N.D.	N.D.	0	2041	Antrum

P.C.A. = Parietal cell antibody.

I.F.A. = Intrinsic factor antibody.

I.F. = Intrinsic factor.

N.D. = Not done.

exogenous intrinsic factor associated with deficient production of intrinsic factor with or without a depressed serum vitamin B_{12} level but without megaloblastic anaemia. In patient 9, the presence of intrinsic factor antibody suggests that the underlying lesion of pernicious anaemia may have been present. Patients 10-13 had parietal cell antibody alone. Two had very low levels of intrinsic factor secretion. From Tables 25 and 26 it is seen that in the 8 cases of pernicious anaemia and latent pernicious anaemia (patients 1-8) the cancer was in the body in 6 cases and the antrum in 2.

Serum vitamin B_{12}

Table 27 shows four patients who had serum vitamin B_{12} levels below the lower limit of normal, but who had neither parietal cell antibodies nor intrinsic factor antibodies. None was known to have pernicious anaemia : two were achlorhydric but one of these absorbed vitamin B_{12} normally. No inhibitors to the growth of Euglena gracilis were found in the serum of any of these patients. The curves obtained for two such patients are shown in Fig. 17. Patient No. 1 with gastric carcinoma had a serum vitamin B_{12} level of 48 μμg/ml. and patient No. 2 had a level of 168 μμg/ml. 0.1 ml. of serum from these patients added to each tube produced no inhibition. Inhibition could not have been masked by the vitamin B_{12} in the serum of Patient No. 2 since this represents the addition of only 0.42 μμg/ml. per tube.

Table 27. Gastric Carcinoma patients with low serum vitamin B_{12} levels.

No.	Age	Sex	Vitamin B_{12}		Stomach		Site
			Serum (pg/ml)	Absorption*(%)	Acid	I.F.**	
14	65	F	112	N.D.	N.D.	N.D.	Body
15	64	M	119	19	0	N.D.	Body
16	68	F	139	N.D.	N.D.	N.D.	Antrum
17	61	M	144	N.D.	0	N.D.	Body

* Schilling method.

** = Intrinsic factor.

N.D. = Not done.

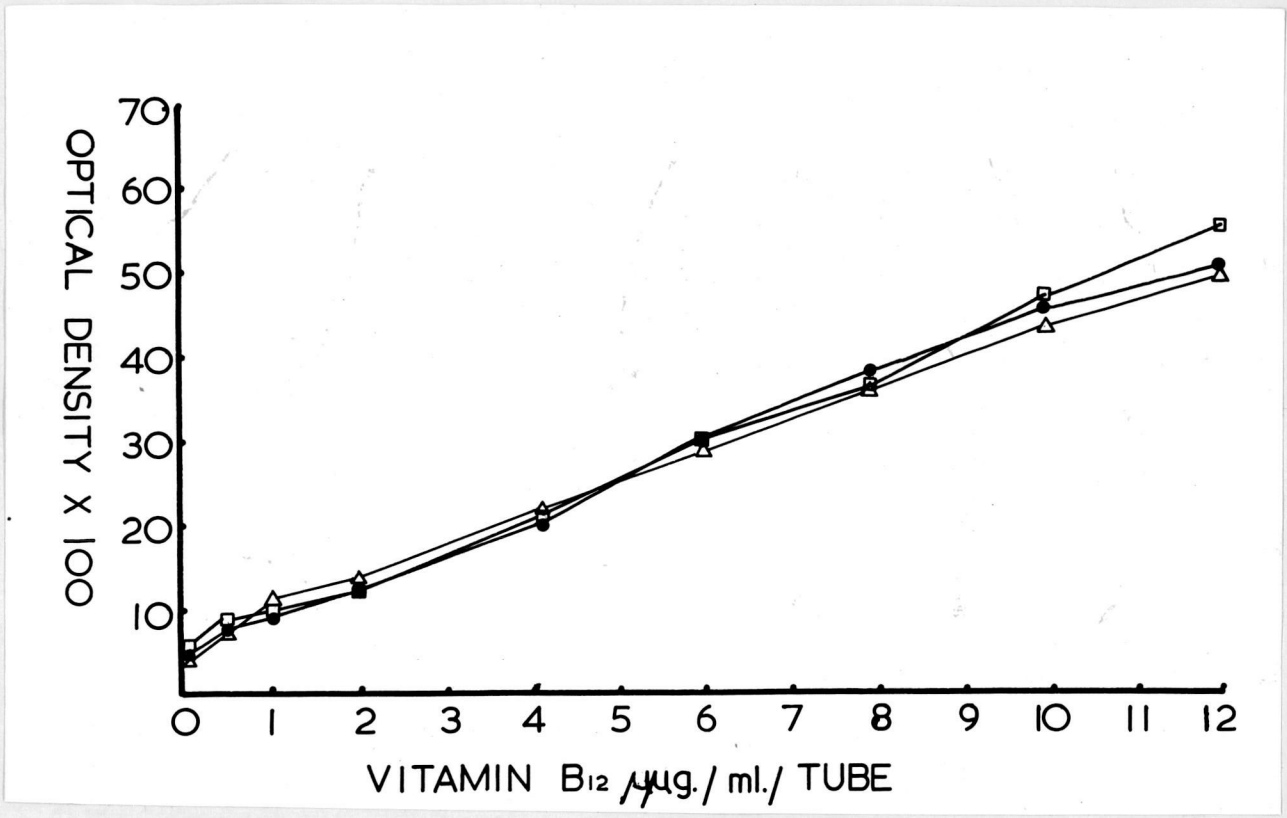

Figure 17. Studies for the detection of serum inhibitors. The growth of Euglena gracilis at varying concentration of vitamin B_{12}, with the addition of the following sera.

● Normal serum.

□ Serum from a patient with gastric cancer and a serum vitamin B_{12} level of 48 µµg/ml.

△ Serum from a patient with gastric cancer and a serum vitamin B_{12} level of 168 µµg/ml.

OESOPHAGEAL CARCINOMA

Associated diseases

These are listed in Table 28. It will be seen that 8 patients had had a previous iron deficiency anaemia and 4 of these patients fall into the group of upper oesophageal carcinoma. Only one patient had achalasia and one had pernicious anaemia. Six patients had had a peptic ulcer proven at some time in their life, one of which was gastric in site. Several other patients had epigastric pain and discomfort but barium studies had not been performed. Eight other patients had operations for peptic ulceration at some time. Five patients had thyroid disease.

Oesophageal carcinoma and operation for peptic ulcer

There was a 9% incidence of previous operation for peptic ulcer and the details of these cases are shown in Table 29. The interval between the operation and the development of the carcinoma varied from 6 years to 40 years with a mean of 19 years. The ages of the patients at the time of diagnosis of the cancer were not significantly different from the ages of the whole group of 92 patients. It will be seen that 6 of the 8 patients had tumours of the lower or mid oesophagus. Other clinical and haematological findings in these 8 cases are given below.

Patient 1 : This patient had hyperthyroidism treated in 1931 by radiotherapy of the age of 29. Subsequently in 1956, she developed carcinoma of the breast which was treated by mastectomy and radiotherapy. At the time of diagnosis of her oesophageal tumour in 1958, she was mildly

Table 28. Disease associations in 92 cases of squamous oesophageal carcinoma.

	Number
Previous iron deficiency anaemia	8
with dysphagia	2
Paterson-Kelly syndrome	1
Diabetes	3
Achalasia	1
Pernicious anaemia	1
Hiatus hernia	2
Peptic ulcer	6
Operation for peptic ulcer	8
Other tumours*	5
Thyroid disease	5

* Two patients had carcinoma of breast, one had bladder papilloma, one had lip epithelioma and one had rectal carcinoma.

Table 29. The development of squamous carcinoma of the oesophagus after gastric surgery.

Patient	Sex	Age	Ulcer Site	Operation	Distance from incisors (cm.)	Years between surgery and tumour
1	F	65	Duodenal	Gastroenterostomy & vagotomy	19	7
2	M	61	Duodenal	Partial gastrectomy (Polya)	19	19
3	F	64	Duodenal	Partial gastrectomy (Polya)	28	17
4	M	67	Gastric	Partial gastrectomy (Polya)	32	13
5	M	66	Duodenal	Gastroenterostomy	40	39
6	M	60	Gastric	Partial gastrectomy (Bilroth I)	39	6
7	M	52	Duodenal	Partial gastrectomy (Polya)	35	11
8	M	74	Duodenal	Gastroenterostomy	32	40

anaemic with Hb. 12.5g%. Serum vitamin B_{12} was normal at 304 µµg/ml. but her serum iron was low at 45 µg/100 ml. with an iron binding capacity of 495 µg/100 ml.

Patient 2 : At the time of diagnosis of the oesophageal tumour, this patient was mildly anaemic with Hb. 13.0g%. Serum vitamin B_{12} was very low at 32 µµg/ml. and serum iron was also low at 25 µg/100 ml. with an iron binding capacity of 360 µg/100 ml. A Schilling test gave a urinary excretion of 5% in 48 hours, but an Azure A result of 1.4 suggested that the patient may have been able to secrete acid.

Patient 3 : At the time of the diagnosis of the tumour this patient had Hb of 12.6g%, a serum vitamin B_{12} of 208 µµg/ml. and a serum iron of 90 µg/100 ml with an iron binding capacity of 435 µg/100 ml. The absorption of vitamin B_{12} was normal (25% on Schilling test) and the azure A test gave an equivocal result.

Patient 4 : Two years before the diagnosis of oesophageal cancer, this patient had an iron deficiency anaemia which was treated by his own General Practitioner. At the time of diagnosis of the tumour he was again anaemic with Hb. 10.6g%. Serum vitamin B_{12} at that time was 210 µµg/ml. and serum iron 30 µg/100 ml. The serum folate was 3.9 µµg/ml. One month after completion of radiotherapy, this patient was admitted to another hospital with Hb. 22% and total white count 2,800 (70% neutrophils). The peripheral blood film showed macrocytes and the marrow was megaloblastic. The assay of serum vitamin B_{12} was recorded as normal.

Patient 5 : This patient had a gastroenterostomy for duodenal ulcer 39 years prior to the development of the tumour. One year prior to the tumour, a vagotomy was performed. At the time of diagnosis of the tumour, the patient was not anaemic (Hb 13.9g%) and the serum vitamin B_{12} was 1029 µµg/ml. The serum iron was 85 µg/100 ml.

Patient 6 : At the time of diagnosis of the tumour, this patient had Hb. 11.8g%, serum vitamin B_{12} 197 µµg/ml, serum iron 75 µg/100 ml.

Patient 7 : This patient had a duodenal ulcer diagnosed in 1943. In 1955 it perforated (treated by simple closure) and the patient had an elective partial gastrectomy in 1957. Haemoglobin at the time of the diagnosis of the tumour was 13.4g%. It is probable that the patient was dehydrated at this time as the Hb. was 11.4g% one month previously and a blood film had features of iron deficiency. Serum vitamin B_{12} was 452 µµg/ml. and serum iron 80 µg/100 ml.

Patient 8 : This patient had had a gastroenterostomy for duodenal ulcer 40 years prior to the development of the tumour. Prior to the gastroenterostomy, she had been troubled by vomiting and heartburn for 11 years and there had been haematemesis on one occasion. At the time of diagnosis of the tumour, Hb. was 13.0g%, serum vitamin B_{12} 248 µµg/ml. and serum iron 75 µg/100 ml. The histology of this tumour had some of the features of an adenocarcinoma but if this was so, both the X-ray (Fig.18) and the oesophagoscopy showed normal oesophageal tissue between the tumour and the stomach.

Figure 18. X-ray of barium swallow examination on Case 8 Table 29, showing a tumour in the lower oesophagus.

Oesophageal carcinoma and thyroid disease

Five patients had thyroid disease (Table 30). Patient No. 1 is the same patient as Patient No. 1 in Table 29. Patient No. 2 presented with thyrotoxic heart disease which was controlled only when methylthiouracil was given. Patient No. 3 had parietal cell antibody, was achlorhydric to pentagastrin and had a normal serum vitamin B_{12} level. The serum iron was low at 40 µg/100 ml. Patient No. 4 had received cyanocobalamin injections for 8 years prior to the development of the oesophageal tumour although there is no documentation of the diagnosis of pernicious anaemia. However, when the tumour was diagnosed, he had both antibodies to parietal cells and intrinsic factor, the serum vitamin B_{12} was very low at 72 µµg/ml. and he was anaemic with Hb. 11.3g%. At the same time. clinically he was suffering from hypothyroidism and the protein bound iodine estimation was less than 1 µg/100 ml.

It will be seen from Table 30 that the interval between the development of the thyroid disorder and the tumour varied from 3 to 37 years with a mean of 15 years. The mean age of this group of patients was not significantly different to that of the whole group of 92 patients.

Table 30. Thyroid disease in patients who developed squamous carcinoma of the oesophagus.

Patient No.	Sex	Age	Age at diagnosis of thyroid disease	Thyroid disease	Treatment
*1	F	65	28	Thyrotoxicosis	Radiotherapy
2	F	78	69	Thyrotoxicosis	Methylthiouracil (4 yr.)
3	F	75	64	Thyrotoxicosis	Carbimazole (3 yr.)
**4	M	79	76	Hypothyroidism	Thyroxine
5	F	70	55	Hypothyroidism	Thyroxine

* Patient also had a partial gastrectomy.

** Patient received vitamin B_{12} injections for 18 years and had antibodies to intrinsic factor and parietal cells.

Oesophageal carcinoma and antibodies to parietal cells and intrinsic factor

The clinical investigations on patients with antibodies to parietal cells are shown in Table 31. Sixteen out of 92 patients had parietal cell antibody (17%) and the incidence was approximately 17% for patients with either upper, middle or lower oesophageal tumours. 4/46 male patients had antibodies to parietal cells (8%) and 12 out of 46 female patients had antibodies to parietal cells (27%). The mean age of the group of patients with parietal cell antibodies was not greater than the group of 92 patients as a whole. Three patients had antibody to intrinsic factor, patient No.1 almost certainly secreted acid (see Azure A test) and patient No. 14 did secrete acid. Only patient No. 12 had pernicious anaemia as evidenced by the low serum vitamin B_{12} level. Patient No. 10 had had the Paterson-Kelly Syndrome for several years. She was achlorhydric to pentagastrin but had a normal serum vitamin B_{12} level and a normal absorption of vitamin B_{12}. One other patient (No. 11) had had mild continuous dysphagia for 20 years.

Four other patients were potential achlorhydrics on assessment by the azure A test (0.07, 0.05, 0.11 and 0.08) but did not have gastric antibodies. Three had a normal serum vitamin B_{12} level but the fourth had a serum vitamin B_{12} level of 52 µµg/ml. The haemoglobin at this time was 13.0g%.

Considering the whole group of 92 patients, 41 were iron deficient at the time of diagnosis of the tumour. The incidence of long-standing iron deficiency was difficult to assess because of inadequate documentation but

No.	Age	Sex	Antibodies		B_{12}	Iron	Azure A	Acid	Clinical features
			PCA	IFA					
1	77	F	++++	19	364	85	6.6	-	-
2	53	F	+++	-	164	90	5.0	-	Intermittent mild iron deficiency anaemia
3	78	F	++	-	144	25	-	-	Mother had pernicious anaemia
4	75	F	+++	-	284	40	-	0	Thyrotoxicosis
5	61	M	+++	13	392	140	-	8	-
6	74	F	+++	-	232	60	-	-	-
7	74	F	+++	-	496	70	-	-	-
8	46	F	+++	-	328	40	1.3	-	-
9	55	M	++++	-	248	85	2.0	-	-
10	60	F	+	-	500	45	-	0	Paterson-Kelly
11	70	F	+++	-	300	70	-	-	Documented evidence of iron deficiency anaemia over 7 years. Some dysphagia over 20 years.
12	79	M	+++	13	72	35	?	-	Hypothyroid. Pernicious anaemia
13	76	F	+++	-	96	100	0.25	-	Not anaemic (Hb. 13.6g%)
14	76	F	++++	-	144	105	0.5	-	-
15	68	M	+++	-	276	35	3.3	-	-
16	75	F	++++	-	212	105	0.08	-	-

Table 32: **Details of patients with antibodies to parietal cells or intrinsic factor.**

Table 31.

there were probably 8 cases, 4 of which occurred in patients with upper oesophageal tumours. The incidence of iron deficiency at the time of diagnosis of the tumour was not greater in those with upper oesophageal carcinoma than in the group as a whole.

DISCUSSION

Studies of gastric secretion

The present study confirms that there is a high incidence of achlorhydria in patients with gastric carcinoma even when maximal doses of histamine or pentagastrin are used. Twenty-four of the 55 patients tested in the present series were achlorhydric. Furthermore, there was a high incidence of hypochlorhydria amongst those who could secrete acid, 25 of 31 patients producing less than 10 mEq. in the post-stimulation hour. Acid and intrinsic factor production was significantly less than in patients with benign gastric ulceration or with no gastric disease. Fifty patients had tumours which could be classified as of body or of antrum, and when these were analysed there were no statistically significant differences in secretion of acid between the groups for either males or females. Nevertheless, it is perhaps worth noting that of the 6 patients who secreted over 10 mEq. in the post-histamine hour, 5 had antral tumours. In no case was a secretion of more than 10 mEq. associated with pyloric obstruction, and the one patient with an acid output above the normal range (35 mEq.) had a very small histologically proven cancer with no evidence of pyloric stenosis. Analysis of intrinsic factor production in patients with antral and body tumours showed no significant difference between the antral and body tumour groups and the total amounts of intrinsic factor secreted are similar to those recorded by other authors (Rodbro, Christiensen, Schwartz 1965, Rodbro 1967).

The higher incidence of achlorhydria in body carcinoma could be explained by pre-existing gastric atrophy or by tumour destruction of

parietal cells. The former receives support from the work of Comfort, Kelsey and Berkson (1948), Hitchcock, Sullivan and Wangensteen (1955) and Berkson, Comfort and Butt (1956) who showed that hypochlorhydria and achlorhydria could exist for up to 25 years before the occurrance of neoplasm, but it must be remembered that many of the tests performed at that time do not fulfill present day criteria. It is more difficult to account for achlorhydria with antral tumours. It may be that pre-existing gastric atrophy predisposes to cancer anywhere in the stomach : on the other hand, there is no adequate study of the parietal cell mass in these patients, and parietal cells might exist but fail to function because of destruction of the antral area which produces gastrin. It was decided that study of intrinsic factor secretion in cases of gastric carcinoma would be of value because of the independent secretion of acid, pepsin and intrinsic factor by the human stomach (Poliner and Spiro 1958), because of the relationship between pernicious anaemia and gastric carcinoma and also because of reported abnormalities of vitamin B_{12} absorption in gastric carcinoma. In previous reports of intrinsic factor secretion estimated by a guinea-pig ileum method and given as a percentage of the normal value, 11 cases were recorded (Nabet, Wolff and Besancon 1964). In 10 of these it was found to be depressed. Rødbro, Christiansen and Schwartz (1965) found low acid and intrinsic factor outputs after an augmented histamine test in 7 cases but in 6 of these, unspecified operations had been carried out : in this latter investigation a modified version of the method of Herbert, Gottlieb, Lau and Wasserman (1964) was used for the direct assay of

gastric intrinsic factor. In the present studies, 37 cases had intrinsic factor estimated and there was a significant depression of its production. In addition, certain individuals showed an intrinsic factor secretion more severely affected than acid secretion (Fig. 16). However, experiments designed to look for inhibitors in the gastric juice of patients with gastric carcinoma failed to demonstrate any inhibition.

Antibody studies and pernicious anaemia

Although there is an increased incidence of gastric cancer in established pernicious anaemia, little has been published about the incidence of pernicious anaemia and latent pernicious anaemia amongst patients with gastric carcinoma though low levels of liver vitamin B_{12} and malabsorption of vitamin B_{12} correctable by exogenous intrinsic factor has been demonstrated. (Nelson and Howe 1963). In the present series, 4 patients had been known to have pernicious anaemia for up to 15 years before the occurrence of the cancer, and were receiving regular treatment with vitamin B_{12} - they constituted 5.7% of the 70 patients. One of these patients had neither parietal cell nor intrinsic factor antibodies.

One other patient presenting with gastric carcinoma also had overt pernicious anaemia with a megaloblastic anaemia and a low serum vitamin B_{12} level. In 3 other patients who were not anaemic there was considered to be sufficient evidence for a diagnosis of latent pernicious anaemia. Two of these were achlorhydric, had low serum vitamin B_{12} levels, and malabsorption of vitamin B_{12} corrected by oral intrinsic factor. Both

had intrinsic factor antibodies, and one had parietal cell antibody. The third was achlorhydric with an output of intrinsic factor well within the range for pernicious anaemia, had parietal cell antibodies and a very low serum vitamin B_{12}. These four patients brought the incidence of pernicious anaemia in this series to 10.4%.

These results are not surprising when the sequence of events in the development of pernicious anaemia is considered. The power to produce gastric acid is lost first, and at some time later, when the secretion of intrinsic factor has fallen to below a critical level (Ardeman and Chanarin 1963) malabsorption of vitamin B_{12} will supervene. At this point in time, there may still be several years supply of vitamin B_{12} in the liver. Thus gastric atrophy and achlorhydria exist for many years, with a possible increased risk of carcinoma, before the development of pernicious anaemia.

In the 8 patients with overt or latent pernicious anaemia the cancer occurred in the body in 6 and the antrum in 2. Similarly for the 50 patients whose tumour site could be classified, the body : antrum ratio was approximately 3 : 1. As the lesion of pernicious anaemia involves severe intestinal metaplasia and gastric atrophy of the body which some consider to be a premalignant state, it is not surprising that body cancers are common. However, gastric carcinoma can also occur in the antrum as it did in two cases described here, which most consider to be histologically normal in pernicious anaemia (Cox 1943, Magnus and Ungley 1938, Meulengracht 1939), though gastritic changes have been reported (Schade 1961).

Five other patients were found to have gastric antibodies without other evidence of pernicious anaemia. In 4, parietal cell antibody alone was present, and in each case the serum vitamin B_{12} was normal. One patient had both parietal cell and intrinsic factor antibody and with regard to the latter it is of interest that the patient was not achlorhydric. The high serum vitamin B_{12} level of 1,000 pg/ml. raises the possibility that this was a false positive intrinsic factor antibody test though both results were confirmed on several occasions. The addition of cyanocobalamin in vitro to give a serum level of 1,000 pg/ml. does not give false positive tests. Most workers consider that the presence of the intrinsic factor antibody is highly suggestive of the underlying lesion of pernicious anaemia (Wangel and Schiller 1966) although there are exceptions (see Section I introduction).

Finally, although it is likely that parietal cell and intrinsic factor antibodies are both late occurrences in evolution of chronic gastritis, antibodies were not found in many patients with severe secretory defects. In particular, parietal cell antibody would have been expected since its presence is correlated with diminished gastric secretory function and chronic gastritis. (Adams, Glen, Kennedy, Mackenzie, Morrow, Anderson, Gray and Middleton 1964, Te Velde, Hoedemaeker, Anders, Arends and Nieweg 1966, Williams, Scott, Beck and Blair 1966, Wright, Whitehead, Wangel, Salem and Schiller 1966). The present results would seem to point either to the fact that in the majority of cases if chronic gastritis does precede the carcinoma it is probably not autoimmune in nature, or, if it is autoimmune in nature these patients, by the time

they develop carcinoma have some abnormality of the immune system whereby the power to produce antibodies is lost. It is noteworthy that even in a case of proven pernicious anaemia in a young patient (case No. 1 Table 25) neither parietal cell nor intrinsic factor antibodies were present at the time of development of carcinoma. These would have been expected. The existence of these cases of gastritis and achlorhydria without antibodies has been confirmed by Kravetz, Van Noorden and Spiro (1967) and it is therefore possible that the defect in immune response in some cases of carcinoma (Lytton, Hughes and Fullthorpe 1964) may be responsible for these findings.

Serum vitamin B_{12} levels

There were four patients who had serum vitamin B_{12} levels below the lower limit of normal and in whom investigations were insufficient to allow a cause to be determined as operative treatment could not be delayed. Two had achlorhydria and one of these had normal vitamin B_{12} absorption. Serum inhibitors to the growth of Euglena gracilis were not found in any patient. The presence of inhibitors in normal serum has been shown (Girdwood 1960, Anderson 1964) but the inhibition present in cases of carcinoma would need to be much greater than that present in normal serum to produce the serum vitamin B_{12} results obtained for these patients.

Oesophageal carcinoma and anaemia

The present study of 92 cases of oesophageal carcinoma was undertaken to re-examine the possible relationships between pernicious anaemia, iron deficiency anaemia and the development of oesophageal cancer. As will be discussed, the study provides little evidence to substantiate the theory that iron deficiency or pernicious anaemia are important associations of oesophageal carcinoma and the supposed association with the Paterson-Kelly Syndrome revealed only one definite case. By contrast, there was a 5% incidence of thyroid disease and a 9% incidence of previous gastric surgery.

Patient Selection

Some degree of patient selection must have taken place since some patients (approx. 10%) were treated initially by surgical resection and were not included in this series. In addition, it is possible that other patients were too elderly or infirm to be referred by their Practitioners or other Physicians for radiotherapy. The present series has a similar mean age (66 years) to previous studies in the Department of Radiotherapy in Edinburgh but the sex ratio is unity in comparison to the male to female ratio of 1:2:1 given by the Registrar General for Scotland for the period 1954-63. However, these latter figures include many adenocarcinomas which were excluded from the present series and they also exclude post-cricoid carcinomas which are certified as death from carcinoma of the 'hypopharynx' (Pearson 1966). Thus the exclusion of post-cricoid cancers, the majority of whom occur in women, from the present series would give a slight predominance of males in the series.

Although it was intended to obtain all the required information at the patient interview, it was discovered that many important events, for example, gastric operations were forgotten by some patients. Thus on every occasion possible, General Practice records and previous hospital notes were consulted. Unfortunately, on many occasions, these were incomplete and inadequate and because of this the incidence of associated disease detected by this survey may be an underestimate.

Patients were excluded from the series if, in the opinion of a pathologist the tumour was an adenocarcinoma. True oesophageal adenocarcinoma

can occur in the oesophagus in areas of ectopic gastric epithelium but there is great difficulty in deciding whether an adenocarcinoma in the lower oesophagus has extended there from the stomach. The histology of some tumours is difficult to classify as either squamous carcinoma or adenocarcinoma particularly when the cells are undifferentiated and so it is possible that the present series of cases contained s small number of cases that arose from glandular epithelium.

All investigations were carried out on patients in the two days prior to radiotherapy treatment. An occasional patient had been transfused prior to transfer to the radiotherapy unit, and in these cases, the haematology results have been omitted. In such cases, the serum iron, serum vitiamin B_{12} and antibody results were accepted as unaltered by transfusion. Azure A tests were carried out because of the difficulties of intubation of the stomach in patients with oesophageal carcinoma. Where further gastric function or absorption tests were required, these were performed after radiotherapy had been commenced.

Carcinoma of the oesophagus and previous gastric operations

Wright and Richardson (1967) noted a 12% incidence of long-standing dyspepsia in 67 patients with cancer of the thoracic oesophagus. Although they do not comment upon it, 4 of these patients had had previous gastric surgery, one for gastric ulcer, two for duodenal ulcer and one for gastric carcinoma. In the present series, there was a 9% incidence of previous gastric surgery. One of the cases (case No.1, Table 29) occurred at the level of the thyroid gland subsequent to thyroid irradiation but six of the other seven occurred in the lower half of the oesophagus. It is possible that this association represents a chance finding and there is no doubt that a much larger series of cases would require analysis before an aetiological relationship is claimed. In addition, if the operation was in some way an aetiological factor in the development of the tumour, then the mean age of these patients might be expected to be less than that of the group as a whole, in the same way that the mean age of post-cricoid carcinoma is less (Pearson 1966). On the other hand, several of the gastric operations were performed at a time when operation for peptic ulcer was much more infrequent than it is now.

In addition, the incidence of 9% is in excess of any of the other established associations of carcinoma of the oesophagus such as achalasia and Paterson-Kelly Syndrome. If there is an aetiological relationship then the mechanism could be through nutritional deficiency or through regurgitation of irritant secretions into the oesophagus.

From the point of view of nutritional deficiency, all but one of the

cases of tumour and previous gastric operation were mildly anaemic, at the time of diagnosis. In addition, one patient had a very low serum vitamin B_{12} level and all patients had a serum iron level at or below the lower limit of normal. Many patients develop regurgitation of gastric and duodenal contents into the oesophagus after partial gastrectomy, this being mainly due to alteration in the oesophago-gastric angle (Windsor 1964). It is possible that if this mechanism was persistent over many years it could lead to oesophagitis and epithelial changes which might predispose to the development of carcinoma. Such irritation would be likely to be maximal in the lower oesophagus and six of the eight tumours were sited here. If this theory was correct, then a high incidence of oesophageal carcinoma might be expected secondary to hiatus hernia but there is no evidence for this although Tanner and Smithers (1961) consider that there may be. On the other hand, a gastric operation usually predisposes to a quantity of bile and pancreatic juice in the stomach or gastric remnant and it is possible that these might effect the oesophageal mucosa in a different manner to acid and pepsin. The situation might be analogous to the possible increased incidence of carcinoma in the gastric remnant after partial gastrectomy. For example, Kuhlmayer and Rokitansky (1954) in a review of necropsy findings in patients who had had a subtotal gastric resection for gastric or duodenal ulcer 10 - 25 years previously, gave an incidence of 11% of gastric cancer in the stump of the stomach, about twice that of the expected incidence. Krause (1958) followed up 361 patients who had had a partial

gastrectomy for ulcer between 1905 and 1933. Twenty-five cases of gastric cancer occurred instead of an expected total of just over 11. However, another large series (Liavaag 1962) was not conclusive for an increase of gastric cancer after partial gastrectomy.

In conclusion, if there is a possible association between oesophageal squamous carcinoma and previous gastric surgery, then it may be due to nutritional deficiency, a cause cited in relation to oesophageal cancer in Africans (Hutt and Burkitt 1965) and in relation to malabsorptive disease in this country (Wright and Richardson 1967), or it could be secondary to regurgitation and mucosal change. It would be desirable to determine the incidence of oesophageal cancer in a larger group of patients with a partial gastrectomy performed many years previously.

The association of oesophageal cancer with thyroid disease

Of the 5 cases of thyroid disease, one (case No. 1) had had irradiation to the thyroid gland and developed oesophageal cancer at this level. The 4 other cases had not had previous radiotherapy. The association between oesophageal cancer and thyroid disease has not been commented upon before but Blendis, Sahay and Kreel (1965) do note an association between cricopharyngeal web and thyroid disorder. In 1938, McGee and Goodwin had commented upon the presence of a bilateral nodular goitre at autopsy in a case of sideropenic dysphagia. Smiley, McDowell and Costello (1963) reported enlargement of the thyroid gland in 4 patients and a previous thyroidectomy in another case, in 27 cases of sideropenic dysphagia. In the 3 cases described by Blendis and Kreel (1965), iron deficiency

anaemia was excluded as a cause as far as was possible. Assuming that a web is simply a reflection of a degenerative mucosa and the latter is more likely to develop carcinoma, then there may be a relationship between oesophageal squamous cancer and thyroid disease. In this respect, it should be remembered that the thyroid gland shares a common origin from the primitive foregut with the buccal, oesophageal and gastric mucosa. It is possible then that these tissues could undergo degenerative changes at approximately the same time. This association of thyroid disease with degenerative disease of the foregut mucosa is already well recognised in some instances, e.g. thyrotoxicosis and glossitis (Means 1948), thyrotoxicosis and achlorhydria (Lerman and Means 1932, Berryhill and Williams 1932, Bock and Witts 1963), myxoedema, gastric atrophy and pernicious anaemia (Lerman and Means 1932, Tudhope and Wilson 1960). These associations will be discussed further.

Antibodies to parietal cells and intrinsic factor in oesophageal carcinoma

Using the immunofluorescent test it is possible to detect parietal cell antibodies in nearly 90% of patients with pernicious anaemia. (Doniach and Roitt, 1964). In patients with pernicious anaemia who are aged less than 60 years, the incidence of parietal cell antibody was higher than those aged over 60 years (Table 32).

The lower incidence in the older pernicious anaemia patients may be due to the advanced state of the gastritic process which has led to gastric atrophy. The incidence of parietal cell antibody in pernicious anaemia in differing geographical populations is similar (Coghill, Doniach, Roitt, Mollin and Williams 1965, Fisher and Taylor 1965, Irvine 1965, Jeffries and Slessenger 1965, Roitt, Doniach and Shapland 1965, Taylor, Doniach, Couchman and Shapland 1962). A raised incidence of parietal cell antibody has been reported in simple atrophic gastritis (Mackay 1964, Wood, Ralston, Ungar and Cowling 1964) and in iron deficiency anaemia. For example Dagg, Goldberg, Anderson, Beck and Gray (1964) found parietal cell antibody in 33% of patients with iron deficiency who were achlorhydric. The interrelationships of pernicious anaemia, iron deficiency anaemia and gastritis are discussed in the section on iron deficiency anaemia, but it is generally accepted that there is a close relationship between these conditions. (McFadyen, Goldberg, Dagg and Anderson 1967). The incidence of parietal cell antibodies in the normal population varies with sex and age. In the series of Doniach and Roitt (1964) it is 2% in young people

Table 32. The incidence of parietal cell antibodies in pernicious anaemia from Roitt and Doniach, 1964.

Patients	Number tested	Percentage positive
Adult P.A. Age <60	69	96%
Controls	42	5%
Adult P.A. Age >60	114	82%
Controls	58	16%

under 20, 6-8% in the age range 30-60 and up to 19% in females over 70 years of age. It is likely that in all these groups, the incidence of parietal cell antibody represents the incidence of marked gastritis (Coghill, Doniach, Roitt, Mollin and Williams 1965, Roitt, Doniach and Shapland 1965, Te Velde, Hoedemaeker, Anders, Arends and Nieweg 1966, Wright, Whitehead, Wangel, Salem and Schiller 1966). Within the geographical region of the present studies, the incidence of parietal cell antibodies in blood donors and in hospital control population has been assessed by Irvine, Davies, Tietelbaum, Delamore and Wynn-Williams (1965). In blood donors, the highest incidence was 13% in the female 50-60 age group (but some anaemic patients were excluded from this series). In the "hospital control" group, the highest incidence was 27% in the 60-70 year old female group.

In the present study, the incidence of parietal cell antibody in patients with oesophageal carcinoma was studied. If a proportion of patients with oesophageal carcinoma had suffered from long-standing iron deficiency, or if there was an increased incidence of atrophic gastritis then a greater incidence of parietal cell antibody than in a control group might be expected. A further control series of patients was not provided since this had been documented for the South-East Region of Scotland (Irvine, Davies, Tietelbaum, Delamore and Wynn-Williams 1965). On this basis, the overall incidence of 17% of oesophageal carcinoma patients with parietal cell antibody does not seem excessive, neither does the female incidence of 27%, bearing in mind that the

average age of all patients was 66 years. The average age of patients with parietal cell antibody was 68 years which was not significantly different from that of the whole group. If the tumour site is considered, there is no difference in the incidence of parietal cell antibody between upper, middle and lower oesophageal tumours. Thus the study failed to show an increased incidence of parietal cell antibody that might provide evidence of an increased incidence of long-standing iron deficiency, gastric atrophy or an increased incidence of pernicious anaemia. It must be argued that the high incidence of iron deficiency in the patients with oesophageal carcinoma is a reflection of poor dietary intake in the period between the onset of the dysphagia and the diagnosis of the tumour.

With the intrinsic factor antibody detecting system of Ardeman and Chanarin (1963), 60% of patients with pernicious anaemia give positive results. Intrinsic factor antibodies are not found in atrophic gastritis without pernicious anaemia (Ardeman and Chanarin 1963, Roitt, Doniach and Shapland 1965) but they have been found occasionally in thyroid disease. In the present study, of the 3 patients with antibody to intrinsic factor, two secreted acid and the significance of antibody in these cases is uncertain. The third patient had pernicious anaemia, the only one in the whole series to definitely have the disorder. Two other patients may have been developing the disorder, 1 had parietal cell antibody and a serum vitamin B_{12} level of 96$\mu\mu$g/ml., the other did not have either antibody but the serum vitamin B_{12} level was 52$\mu\mu$g/ml. No other investigations were performed.

SUMMARY OF SECTION II

Seventy patients with gastric carcinoma were investigated. In 54, pentagastrin or augmented histamine stimulation tests of gastric secretion were performed. Intrinsic factor production was studied in 40 cases. No statistically significant differences were found between patients with body or antral growths with regard to acid or intrinsic factor secretion. However, some patients with antral tumours had a normal or increased acid production. There was no relationship between the secretory findings and the extent of the tumour. Some patients who secreted acid had a low intrinsic factor output but there was no evidence of inhibitors in the gastric juice. The incidence of parietal cell and intrinsic factor antibodies was studied in the 70 patients. In addition to 4 known cases of pernicious anaemia, a further 4 overt or latent cases were found increasing the incidence of pernicious anaemia in the series from 5.6% to 10.4%. In some other patients, the serum vitamin B_{12} level was low for no apparent reason and in these cases serum inhibitors to the growth of Euglena gracilis could not be demonstrated.

Ninety-two consecutive cases of oesophageal squamous carcinoma were studied. Sixteen of the 92 patients had parietal cell antibodies and the incidence was 17% for upper, middle and lower oesophageal tumours. The 27% incidence in female patients was no greater than in a control group. Only one patient had pernicious anaemia. Five of the 92 patients

had suffered from thyroid disease and the interval between the development of the thyroid disorder and the tumour varied from 6 to 37 years with a mean of 15 years. The most frequent disease association was with previous gastric surgery in 8 patients. On average, the gastric operation had been performed 19 years previously and 6 of the 8 cases occurred in the lower oesophagus. The reason for this association could be nutritional deficiency after gastric surgery or on the increased tendency to reflux of gastric or intestinal content into the lower oesophagus.

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